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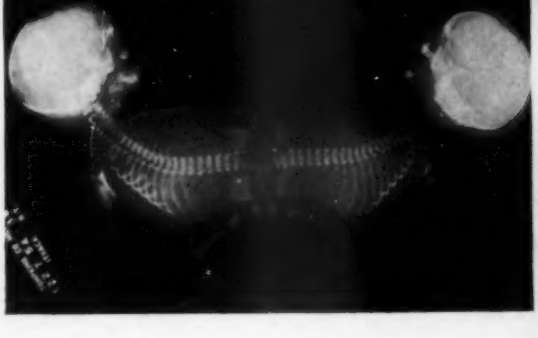

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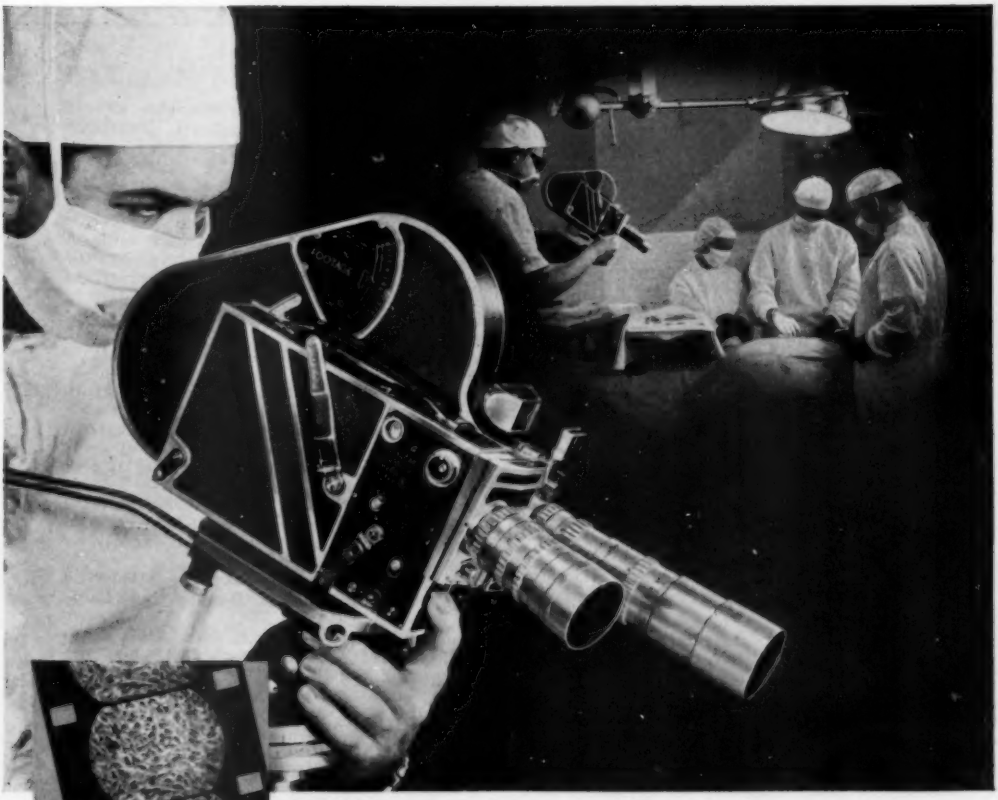

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A.M.A. Archives OF PATHOLOGY

Autopsy Correlation with Clinically Determined Atherogenic Index

XII. Newer Clinical and Laboratory Studies in the Aged

IRVING CHAPMAN, M.D.
A. ALLEN GOLDBLOOM, M.D.
GERALD MIRRER, M.D.
and
HAROLD B. EIBER, M.D., New York

The concept of the primacy of lipids in the pathogenesis of arteriosclerosis derives predominantly from the anatomic investigations of Anitschkow.* With newer physicochemical techniques of analyzing serum lipoprotein levels, Gofman demonstrated a statistically significant correlation of the standard S_f 0-12 and standard S_f 12-400 lipoprotein levels with the degree of arteriosclerosis inferred from clinical evaluation.[3] These data have been further refined by Gofman so as to give relative importance to the standard S_f 0-12 and standard S_f 12-400 lipoprotein levels in the pathogenesis of arteriosclerosis. The resultant index is termed atherogenic index.

Submitted for publication Jan. 26, 1956.
Aided by Sophie D. Cohen and William W. Cohen Foundation.

From the New York Medical College, Metropolitan Medical Center (Bird S. Coler Division).
Pathologist, Bird S. Coler Hospital, and Assistant Clinical Professor of Pathology, New York Medical College (Dr. Chapman); Clinical Professor of Medicine, New York Medical College (Dr. Goldbloom); Assistant Pathologist, Bird S. Coler Hospital (Dr. Mirrer), and Instructor of Medicine, New York Medical College (Dr. Eiber).

* References 1 and 2.

As part of our investigation of various lipid levels in the 80- to 100-year-old age group,[4] blood samples of 100 patients were submitted for S_f fraction determinations. These were all single determinations. The results obtained were at or below levels usually found in the 20- to 29-year-old age group. (See Table 2.)

If we were to accept the theory that the atherogenic index was in some way causally related to the development of arteriosclerosis, one of two possibilities could explain the unexpected low levels found in our elderly patients:

1. That the 80- to 100-year-old group we had examined had always had low levels, and that these persons would have arteriosclerosis of a degree expected in a much younger age group, or
2. That the atherogenic index had previously been higher, had become lower prior to, or at the time of, our examination, and that any severe arteriosclerotic alteration occurred when the atherogenic index was higher.

MATERIAL AND METHODS

Six of the patients studied clinically have come to necropsy. The time interval between the S_f fraction determination and necropsy ranged from two weeks in Case 1 to one year in Case 5. At postmortem examination special attention was given to the aorta and coronary arteries. Gross examination was complemented by histologic examination

TABLE 1.—*Correlation of Degree of Arteriosclerotic Alteration with Clinically Determined Atherogenic Index*

Patients	Sex	Standard S_f 0-12	Standard S_f 12-400	Atherogenic Index	Coronary Arterio-sclerosis	Aortic Arterio-sclerosis
1	M	260	61	37	3+	4+
2	M	262	87	42	3+	2+
3	M	327	118	54	2+	3+
4	F	270	31	32	4+	4+
5	M	200	126	50	3+	4+
6	M	207	149	47	4+	3+

TABLE 2.—*Standard S_f Lipoprotein Levels and Atherogenic Index in Varied Age Groups* *

Age	Standard S_f 0-12	Standard S_f 12-400	Atherogenic Index
Male group			
20-29	302	357	59
30-39	340	474	70
40-49	361	474	74
50-59	376	468	75
60-69	398	441	72
Female group			
20-29	283	234	46
30-39	304	330	51
40-49	324	345	61
50-59	364	424	71
60-69	426	507	84

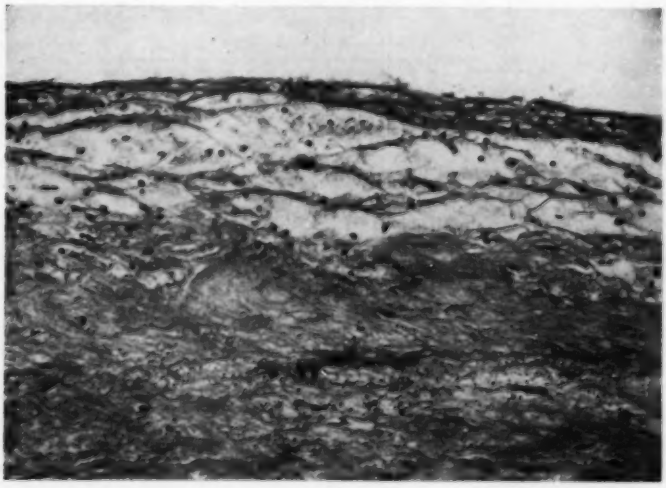
* Gofman, J. W.: Personal communication.

of multiple blocks. These were fixed in 10% formalin, dehydrated in alcohol, cleared in xylene, embedded in paraffin, and cut at 6μ. Companion slides were stained with hematoxylin and eosin, toluidine blue, iron hematoxylin, and orcein Van Gieson. Some of the formaldehyde-fixed tissues were bisected, and frozen sections were stained by Sudan III. Fourteen blocks of tissue were removed from each aorta, and six from each major coronary artery. Approximately 135 slides were examined from each case. A mimeographed diagram of aorta and coronary arteries, with detailed descriptions of the gross alterations in each case, made up part of the protocol. Sites from which tissue was removed for histological examination were noted in the diagram.

All the slides were studied by one of us (I. C.). At the completion of the anatomic study of each case, an over-all grading of the degree of arteriosclerosis of the aorta and coronary arteries was recorded. The grades varied from 0-4, with 0 being the least and 4 the severest.

At the completion of the anatomic study of all the cases, the findings were correlated with the standard S_f 0-12 and standard S_f 12-400 lipoprotein levels, as well as the atherogenic index. The results are charted in Table 1.

For comparison, we have charted the standard S_f values and atherogenic indices for varying age groups in Table 2.



Case 1.—Foam cell collections within the intima as evidence of recent lipid imbibition.

It is evident that the calculated atherogenic index of all of our patients was below the accepted mean for the 20- to 29-year-old age group. From our observations in the six cases, we could not find any correlation between the degree of arteriosclerosis and the atherogenic index. It could, however, be reasoned that the second possible deduction was correct; i. e., that any arteriosclerosis found at postmortem examination could have developed in previous years, when the atherogenic index may well have been higher and that the present rate of arteriosclerotic alteration may either be below or at the 20- to 29-year-old age group.

It is inherent in the concept of lipid primacy in the pathogenesis of arteriosclerosis that the earliest lesions are accumulations of lipid within the subendothelial intima by some imbibition process,[5] and that these lipid collections later elicit fibroblastic reaction with subsequent degenerative changes.[5]

A study of our histological preparations revealed subendothelial intimal foci of foam cells in all of our cases. This differs in degree in the various areas and cases. (See Figure.)

From a morphologic aspect, which only glimpses one moment of the flow of events, this is strong evidence that recent lipid imbibition did occur.

CONCLUSIONS AND SUMMARY

Tissue examination of the aorta and coronary arteries of six autopsied patients in an 80- to 100-year-old age group, who, during life, had standard S_f quotients determined by ultracentrifugal methods, revealed a total lack of correlation between the degree of arteriosclerosis and the atherogenic index.

Despite the markedly low atherogenic index, we find morphologic evidence of recent lipid imbibition as evidenced by foam cell accumulations in the subendothelial intima of all of our cases.

These findings oppose the thesis that the atherogenic index is causally related to the development of arteriosclerosis.

We do not know of any other published autopsy series in persons whose atherogenic index was determined during life.

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Mediastinal Cysts

MURRAY R. ABELL, M.D., Ann Arbor, Mich.

Cysts of the mediastinum are uncommon, but they represent an important group of lesions because their recognition and prompt removal may forestall serious interference with the essential structures of the region. The increased use of roentgenograms for the detection of tuberculosis has resulted in the discovery of many cysts that do not produce symptoms. At the same time, advances in surgery have made the mediastinum a readily approachable region. Such is the combination of circumstances which requires the pathologist to be acquainted with the clinical and structural features of the cysts which occur in the mediastinum.

Derived from 132 patients, 133 surgically removed primary cysts and neoplasms of the mediastinum were examined in the department of pathology at the University Hospital during the 15-year period which ended July 1, 1954 (Table 1). Of this group, 114 lesions were excised completely, and 19 partially. Lesions of endobronchial, endoesophageal, and intrapericardial origin were excluded from this tabulation, as were also neoplasms that arose from the sternum, ribs, or vertebrae, but which projected into the mediastinum. By custom rather than on strict anatomical criteria, neurogenous neoplasms which arose in the paravertebral gutters and projected into the mediastinum were included.

The cysts and neoplasms that were reviewed comprised 15% of all intrathoracic tumors that were excised or from which biopsy specimens were obtained by thoracotomy during the same period, 2% of all intrathoracic specimens obtained by thoracotomy, and 0.11% of the surgical material examined. Because the material was restricted to specimens obtained by surgical means, the lymphoblastomas formed a much smaller group than would have been true if clinical and necropsy material had been assessed. This assumption is supported by Key,[1] who listed 43 lymphoblastomas in a series of 101 unselected mediastinal tumors. The series reported here (Table 1) is in agreement with those of Blades,[2] Curreri and Gale,[3] and Sabiston and Scott [4] in respect to the predominance of neurogenous tumors. The slightly greater number of syncytial-cell tumors of the thymus compared with the number of teratomas conforms with the findings of Sabiston and Scott [4] and is in contrast to the statement of Ackerman and del Regato [5] that the commonest tumor of the anterior mediastinum is the teratoma.

In this report the cysts, but not the cystic teratomas, of the mediastinum are considered. There were 36 cysts excised from 35 patients. They accounted for 27% of the primary mediastinal cysts and neoplasms (Tables 1 and 2) and were second in frequency to the neurogenous group. This is in contrast to

TABLE 1.—*Primary Mediastinal Neoplasms and Cysts*

	No.	Percentage of Group
Neurogenous neoplasms	43	32
Primary cysts	36	27
Syncytial cell neoplasms of thymus	19	14
Teratomas	14	11
Supportive and vascular tissue neoplasms	8	6
Lymphoblastomas	6	5
Miscellaneous group *	7	5
Total	133	100

* This includes 1 aortic body tumor, 1 parathyroid adenoma, 4 thyroid choristomas, and 1 thymic choristoma.

Submitted for publication Feb. 16, 1956.

From the Department of Pathology, University of Michigan.

the statement by Laipply [6] that epidermoid cysts, dermoid cysts, and teratomas together make up the largest group of intrathoracic cysts and tumors, but agrees with the conclusions of Curreri and Gale [3] and Sabiston and Scott.[4] The histopathologic types of cysts and their respective numbers are listed in Table 2.

TRACHEOBRONCHOGENOUS CYSTS

The first report in the American literature of a bronchogenous mediastinal cyst was probably that of Mixter and Clifford,[7] in 1929. Laipply (1945) [6] reviewed the literature, collected reports of 34 cysts, and added 1 case. By 1949, a total of 82 cysts had been reported, and Hardy [8] pointed out that all but 3 were from adults. Curreri and Gale [3] recorded 25 bronchogenous cysts from a series of 145 cysts and neoplasms. In their analysis, they included 109 tumors reported previously by Blades.[2] Groups of 12 and 8 cysts have been reported by Brown and Robbins [9] and by Maier.[10] Maier [10] divided the cysts on the basis of location into five groups: paratracheal, corynal, hilar, paraesophageal, and a small miscellaneous group found in various unusual locations throughout the mediastinum.

TABLE 2.—*Primary Mediastinal Cysts*

Type of Cyst	No. of Cases	Sex		Age		Symptoms
		Male	Female	Actual or Average	Range, in Yr.	
Tracheobronchogenous	17	10	7	Av. 36 yr.	17-56	5
Tracheoesophageal	1	..	1	7½ mo.	.....	1
Esophageal	3	2	1	3 days, 6 wk., 17 yr.	.....	2
Gastroenterogenous	4	4	..	4 mo., 7 mo., 1 yr., 5 yr.	.....	4
Pericardial celomic	8	4	4	Av. 42 yr.	32-49	4
Thymogenous	2	..	2	45 yr., 50 yr.	.....	1
Meningeal	1	..	1	36 yr.	.....	1
Total	36	20	16			18

Clinical Observations.—There were 17 tracheobronchogenous cysts, making this the commonest type in the series (Table 2). The average age was 36 years, with a range from 17 to 56 years. Ten cysts were from male and seven from female patients. Pressure symptoms attributable to the size of the cysts were present in five cases. Two small cysts were incidental findings, observed and removed during operations for other lesions. Most of the cysts were detected on radiographic surveys or examinations for other purposes. Two cysts were known to be present for three years and one for six years. During these periods there was very little increase in size. One cyst was associated with an atelectatic azygous lobe and a separate second cyst of esophageal type. Although arising in the midmediastinum, the tracheobronchogenous cysts usually presented as posterior mediastinal tumors.

Structure.—The majority of the tracheobronchogenous cysts were roughly spherical and unilocular, and they usually contained a grayish mucilaginous material. The largest measured 15 cm. in its greatest diameter. Although usually unicameral, the inner surface often presented a trabeculated appearance due to firmly fixed cartilaginous ribs within the walls. Three cysts were multiloculated, and one consisted of two distinct but firmly adherent compartments. The walls were thin except for the nodules of cartilage and groups of hyperplastic bronchial glands.

Attachments were described for 11 cysts. Eight were attached or adherent to the trachea in the region of its bifurcation or to the right or left main bronchus. Three cysts were related to the right lateral wall of the trachea at a higher level than the others. In one instance the cartilaginous rings were deficient in the area where the cyst was attached to the trachea.

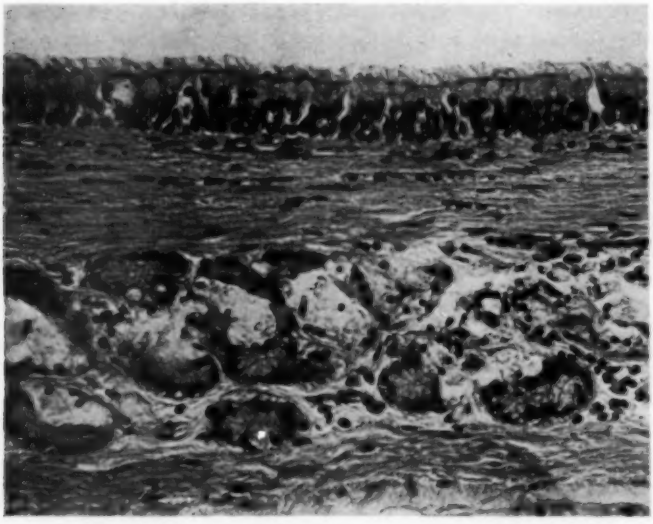
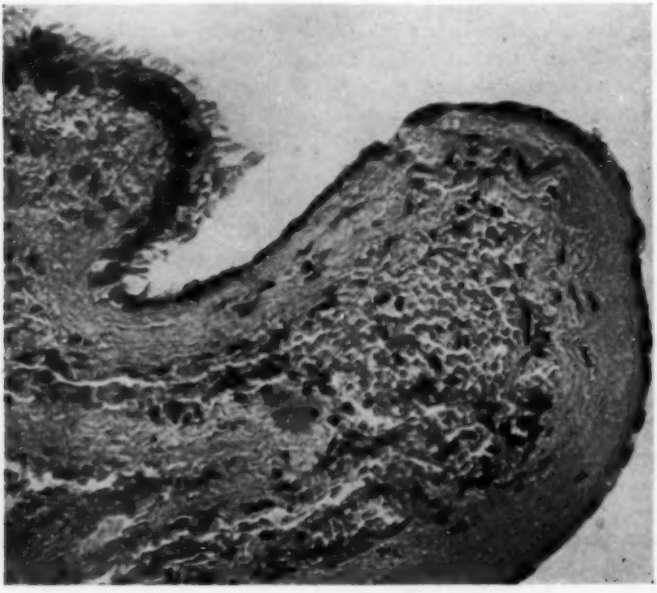


Fig. 1.—Tracheobronchogenous cyst lined by a ciliated pseudostratified columnar epithelium. Bronchial glands beneath smooth muscle and fibrous tissue. Hemalum and eosin; reduced slightly from mag. × 500.

Fig. 2.—Tracheobronchogenous cyst. This shows a transition from ciliated columnar epithelium to a single layer of flattened cells that resemble mesothelium. Hyalinization of stroma beneath the altered epithelium. Hemalum and eosin; reduced ⅙ from mag. × 380.



All cysts of this group were lined by ciliated pseudostratified columnar epithelium (Fig. 1). One possessed stratified squamous epithelium restricted to a small area about the opening of the duct of a bronchial gland. A few cysts showed areas where the mucosa was attenuated and consisted of a single layer of flat or low cuboidal cells that resembled mesothelium (Fig. 2). Beneath epithelium of this type the stroma consisted of hyaline connective tissue (Fig. 2). The walls of all cysts possessed bands and pencils of smooth muscle with perimuscular fibrous stroma (Figs. 1 and 3). Typical bronchial glands were present in 15 cysts, and cartilaginous plates and nodules in 12 (Fig. 3). The bronchial glands often were distended with mucinous secretions and in one instance were dilated sufficiently to be considered cystic. None of the cysts possessed esophageal or gastric elements. Inflammation was limited to a few aggregates of lymphocytes and plasmocytes in the walls of some cysts.

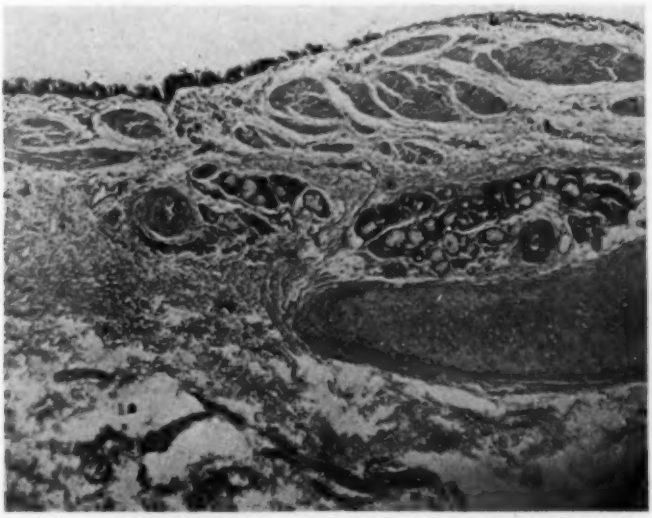


Fig. 3.—Tracheobronchogenous cyst. Part of the mucosa is visible. Beneath it there is fibrous connective tissue with bundles of smooth muscle fibers. Bronchial glands are beneath the muscle but superficial to a cartilaginous plate. Hemalum and eosin; reduced slightly from mag. × 50.

Comment.—The first indication embryologically of a respiratory system is a medial ventral outgrowth, the laryngotracheal groove, which appears at the level of the posterior pharyngeal pouches during the fourth week of intrauterine development.[11] This is converted into a tubular process that parallels the digestive tube. The caudal end becomes enlarged and bifurcates at about the sixth week, forming the primitive lung buds. The mucosa and bronchial glands are entodermal in origin. The cartilage, muscle, and connective tissue are formed from mesenchyme about the entoderm.

The tracheobronchogenous cysts duplicate the structure of trachea or bronchus and are developmental in origin. It is thought that a group of entodermal cells or a diverticulum from the laryngotracheal groove may become sequestrated, carried caudally, and develop into a tracheobronchogenous cyst. If this occurred when the tracheal groove first appeared, the cyst so formed could possess tissues that resembled trachea or bronchus and esophagus. Such cysts have been reported, but they are much less frequent than those of the pure tracheobronchogenous type. Because of the usual absence of elements of the digestive tract in cysts of this group and

the frequent attachment to either trachea or bronchus, it is logical to assume that they, and thus tracheobronchogenous cysts in general, arise at a latter period after the separation of the respiratory from the primitive intestinal system. The report by Arce [12] of a diverticulum of the right primary bronchus in the region where many bronchogenous cysts are found supports this contention. Womack, in a discussion of Blades' paper,[2] stated, "I think we have enough evidence now to show that these tumors, for the most part, represent the abnormal development of supernumerary lungs which takes place very early in embryonic life."

Of particular interest in this series were the three right paratracheal cysts, one of which was associated with a defect in the cartilaginous rings of the trachea at the point of attachment. Maier [10] pointed out that this is the area where the first lateral bronchial branch is formed in some animals. Such a bronchus and, hence, such cysts are rarely found on the left side. This is the location where accessory paratracheal lungs and tracheal diverticula have been found.* We recently observed a cyst and a diverticulum in this location as an incidental finding at necropsy. The diverticulum communicated with the trachea by a very narrow lumen. Fallon, Gordon, and Lendrum [13] suggested the term "lung bud cyst" for cysts of the tracheobronchogenous type.

The observation that tracheobronchogenous cysts usually do not produce symptoms or are not discovered until adult life [8] does not detract from the developmental hypothesis. Their growth is slow, and symptomless cysts have been observed for years with very little increase in size. Necrosis, ulceration, and inflammation are of little importance in these cysts, and the structural features are so characteristic that histologic diagnosis is not a problem.

TRACHEOESOPHAGEAL CYSTS

It is reasonable to expect that sequestration of entodermal cells in the region of the primitive laryngotracheal groove would result in a cyst containing tracheal and esophageal elements. It is also conceivable that a tracheoesophageal fistula could become closed off at each end and a mixed cyst result, which could then be termed a tracheoesophageal cyst. Some of the paraesophageal bronchogenous cysts that have been reported [10] may be of this type. Bremer [16] discussed a cyst arising from the esophagus, which he believed "just missed being" a tracheoesophageal fistula.

Clinical Observations.—The one cyst which was considered to be of tracheoesophageal type was from a girl, aged 7½ months. It was located between the trachea and the esophagus in the superior mediastinum. Symptoms were attributed to pressure.

Structure.—The cyst was unilocular, contained a mucilaginous fluid, and measured 3 cm. in its greatest diameter. It was lined by ciliated pseudostratified columnar and stratified squamous epithelium. Bronchial glands were present. The wall varied in thickness and in some areas resembled that of a tracheal cyst. There was one small focus of cartilage, but other portions of the wall consisted of a thick layer of smooth muscle which was divisible into two layers, resembling those of the esophagus. No striated muscle fibers were identified. Glands beneath the squamous mucosa were interpreted as modified esophageal glands.

Comment.—The tracheal elements were obvious in this cyst. The stratified squamous epithelium, absent in the group of tracheobronchogenous cysts, and the thick muscularis were thought to justify the designation tracheoesophageal. Similar "mixed" cysts were referred to by Laipply [6] and reported by Guillery [17] and Adams and Thornton.[18] Cassel, Cunningham, and Weisel [19] reported a cyst which they thought contained respiratory and gastric elements. In the absence of bronchial glands and cartilage, ciliated epithelium may not indicate a respiratory component. Ciliated epithelium is found in the fetal and adult esophagus,† and sometimes

* References 13 and 14.

† References 11 and 20-22.

is observed in intra-abdominal gastrogenous cysts.[23]

The cyst reported here was thought to have arisen during the early formation of the tracheal groove. This accounts for the combination of respiratory and esophageal elements.

ESOPHAGEAL CYSTS

Laipply [6] stated in reference to the existence of esophageal cysts with structures simulating normal esophagus, "It seems likely from the developmental standpoint that such cysts could occur in the mediastinum. Nevertheless, none of those reported in the literature has been so classified." Evans,[23] however, referred to a cyst that was reported by Hebb,[24] in 1898. It was attached to the esophagus and had a thick muscular wall with "striped and unstriped" muscle. Patterson,[25] in 1934, described an esophageal cyst which was characterized by a "considerable amount of skeletal muscle."

Ranström [20] collected 20 examples of ciliated epithelial cysts of the esophagus and added 2 cases. Gledhill and Morrow [26] found only two reports of such cysts that had been resected from the wall of the esophagus and added a third case. Evidence has been presented which indicates that the cysts arise from a failure of coalescence in a longitudinal axis of vacuoles that appear in the solid stage of the alimentary tube, so that the continuity of the lumen is not established normally.‡ On the other hand, Baar and d'Abreu [14] regarded esophageal cysts as similar in mode of development to accessory lungs, but this opinion is not shared by most authors.

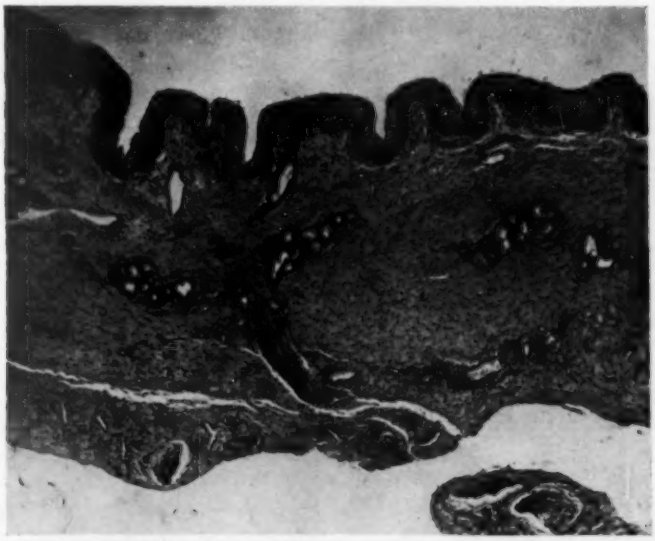


Fig. 4.—Esophageal cyst, lined by moist stratified squamous epithelium. The wall is thick and consists of smooth muscle and fibrous tissue in which esophageal glands are embedded. Hemalum and eosin; reduced slightly from mag. × 50.

Clinical Observations. — This group included three esophageal cysts (Table 2). Two were in the superior mediastinum and were intimately related to the esophagus. Both were from male patients, ages 6 weeks and 17 years. In the latter patient there was also a separate bronchogenous cyst. The third cyst, from a 3-day-old girl, was an incidental finding and was removed at the time of repair of a tracheoesophageal fistula. It

‡ References 20, 26, and 27.

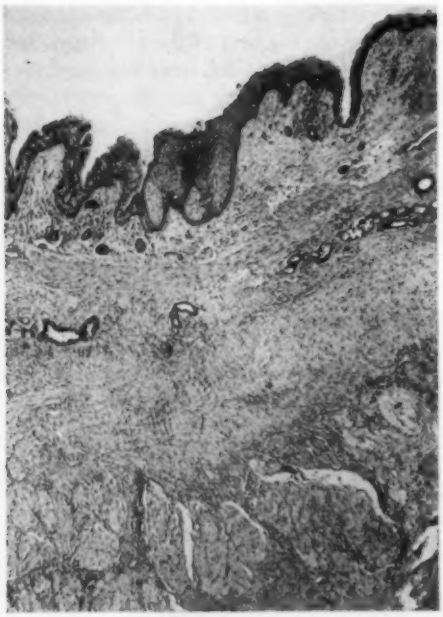


Fig. 5.—Esophageal cyst, Type I. It is lined by moist stratified squamous epithelium which is supported by a lamina propria. The wall consists of a mixture of smooth and skeletal muscle. There are a few esophageal glands. Hemalum and eosin; reduced ⅓ from mag. × 75.

measured 1.7 cm. in diameter and was attached to the lower atretic end of the upper esophageal segment.

Structure.—The three cysts were unilocular and contained mucilaginous material. The two cysts from the male patients were lined by noncornifying stratified squamous epithelium (Figs. 4, 5, and 6), but showed also small areas of ciliated columnar epithelium. A muscularis mucosae was clearly visible in some areas. Esophageal glands extended into the muscularis (Figs. 4, 5, and 6). The latter tended to be arranged in layers, but the most striking feature was the intermingling of striated and smooth muscle (Figs. 5 and 6). The third cyst was lined by ciliated epithelium (Fig. 7) and possessed an ill-defined muscularis mucosae and a thick muscularis propria. No striated muscle fibers or esophageal glands were present.

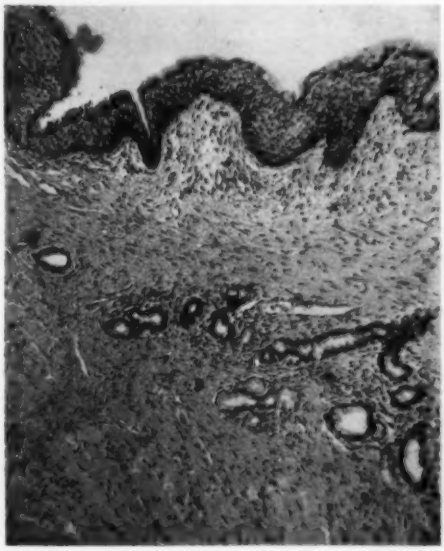
Comment.—The two cysts that had striated muscle in their walls resembled those reported by Hebb [24] and Patterson.[25] They are considered to represent "pinched-off" congenital diverticula. The presence of ciliated epithelium does not imply a bronchogenous component since it is present in the fetal esophagus and may persist after birth.§ The third cyst was similar to those reported by Ranström.[20] Because of its location, the formal genesis of this cyst may have been related to the development of a tracheoesophageal fistula rather than to persistent vacuoles in the primitive alimentary tube, as suggested by Ranström [20] and others.‖ Thus, two distinct types of esophageal cysts can be recognized; and, of each, the formal genesis is probably different. The more characteristic type resembles adult esophagus and possesses a moist squamous mucosa and striated muscle in its wall. The second type is lined entirely by ciliated mucosa, which resembles that of the fetal esophagus.

GASTROENTEROGENOUS CYSTS

The first report of mediastinal gastrogenous cysts, as such, in the English literature

§ References 11 and 20-22.
‖ References 26 and 27.

Fig. 6.—Esophageal cyst, Type I. Prominent skeletal muscle forming part of the wall. Hemalum and eosin; reduced about ¼ from mag. × 125.



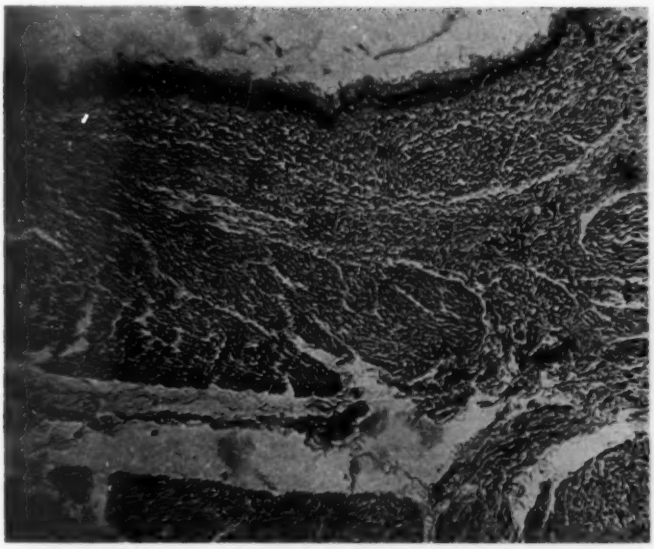


Fig. 7.—Esophageal cyst, Type II. The lining is ciliated pseudostratified columnar epithelium. The wall is thick and composed of smooth muscle, which shows a tendency to be separated into layers. Hemalum and eosin; reduced slightly from mag. × 190.

was that of Mixter and Clifford, in 1929.[7] They stated: "We have failed to find mention, in the literature, of the occurrence of the gastrogenic type of cyst of the mediastinum." They reported two cysts in male infants, one of which was excised successfully. Linder [28] stated that the first cyst of this type was reported by Staehelin-Burckhardt, in 1909.[29] Before [30] and after [31] recognition of gastrogenous cysts by Mixter and Clifford,[7] such cysts were recorded in the literature as teratomas or dermoids. Similar cysts have been reported under a variety of names, such as gastroenteric cysts,¶ gastric cysts,# esophageal duplications,* enterogenic cysts,† archenteric cysts,[39] duplications of the foregut,[14] gastrogenous cysts,[40] gastrogenic cysts,[41] and enterocystoma.[42]

Ladd and Scott,[35] in 1944, reported five mediastinal cysts of enteric origin. Four were from baby boys and one from a baby girl. Laipply [6] accepted 12 gastrogenous and 3 enterogenous cysts in the literature until January, 1944, and reported an additional example. Olken [32] reviewed the findings in 18 examples of gastroenteric cysts and reported 1 gastric cyst. Twelve of the cysts were lined primarily by gastric mucosa, whereas the others were lined primarily by intestinal mucosa. Surgical excision was successful and recovery complete in only three cases. Of these cysts, 75% were discovered within the first year of life. Williams and Johnson (1952)[34] collected 32 "mediastinal gastric cysts" from the literature and recorded 1 case.

The number of reviews and reports of gastroenterogenous cysts belies their comparative rarity. Sabiston and Scott [4] found only 2 gastroenterogenous cysts in a series of 101 cysts and neoplasms. Blades' [2] series of mediastinal tumors did not include a single cyst of this type, whereas there were 23 bronchogenous and 10 pericardial cysts, and 1 esophageal cyst. This ratio could be anticipated, however, since his material was

¶ References 28 and 32.

References 33 and 34.

* References 35 and 36.

† References 37 and 38.

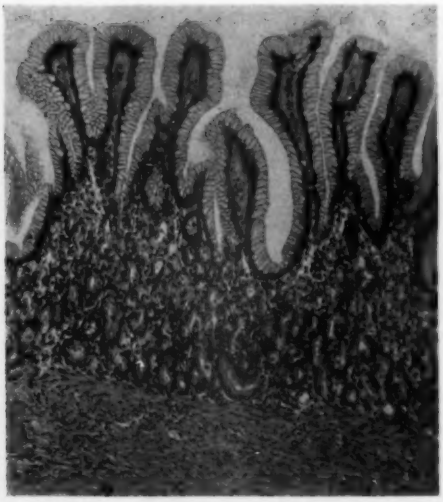


Fig. 8.—Mucosa of a gastroenterogenous cyst. The surface epithelium consists of columnar mucus-secreting cells. The glands of gastric type contain both chief and parietal cells. Hemalum and eosin; reduced about ¼ from mag. × 160.

from adults, and gastroenterogenous cysts are found predominantly in infants; however, Schwarz and Williams [83] reported one from a 23-year-old man, and Cassel, Cunningham, and Weisel,[19] one from a 24-year-old man.

Clinical Observations.—Four cysts of this series were of the gastroenterogenous type (Table 2). They were resected from boys whose ages were 4 months, 7 months, 1 year, and 5 years. All were located in the posterior mediastinum and produced symptoms due to pressure. Radiographic studies revealed abnormalities of the cervical and/or dorsal spinal column in all patients. In three instances there was lack of fusion, resulting in the formation of hemivertebrae. There was a congenital mid-dorsal scoliosis in the fourth patient. Of interest were the descriptions of attachments and intimate relationships of the cysts to the thoracic vertebrae, as supplied by the operating surgeons: "Attached by a short pedicle to the region of the third left intervertebral dorsal foramen"; "to the lateral and anterior surfaces of the bodies of the vertebrae and particularly to the intervertebral foramens"; "very dense attachment which seemed to come from behind the third and fourth dorsal vertebrae," and "by a pedicle at the level of the 4th rib posteriorly." One patient also had a tubular duplication of the terminal ileum, which was resected successfully. The resections of the four mediastinal cysts were uncomplicated, and the results were excellent.

Structure.—Three cysts were roughly spherical and unilocular. The largest measured 9 cm. in diameter. The fourth cyst consisted of two communicating compartments. The walls varied from 3 to 6 mm. in thickness. The most characteristic histologic feature, present in all four, was a thick muscularis, which resembled that of stomach or of intestine and was divisible into either two or three fairly distinct layers (Fig. 9). A muscularis mucosae was found in all cysts but was not present in every section. Nerve fibers and ganglia which corresponded to Auerbach's plexus were

Fig. 9.—Another region in the wall of the same gastroenterogenous cyst shown in Figure 8. The mucosa here consists of a single layer of columnar cells, and there are no gastric glands. The muscularis is in two definite layers. Hemalum and eosin; reduced ⅓ from mag. × 200.

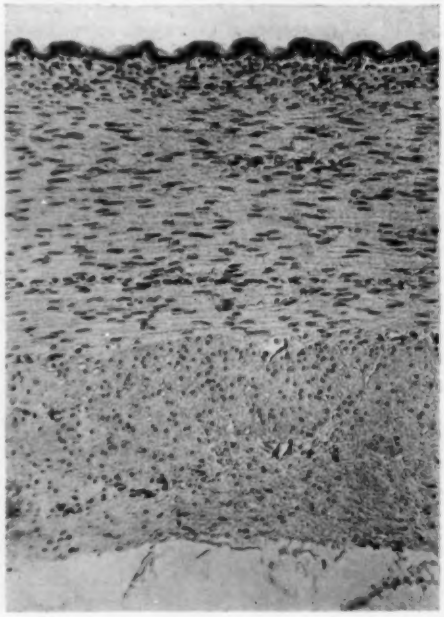
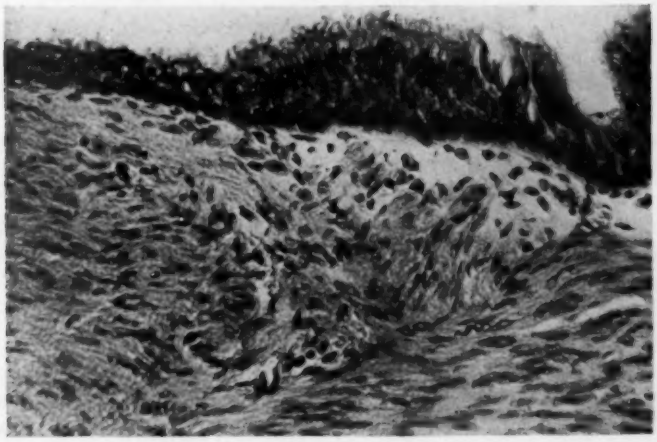


Fig. 10.—A group of sympathetic ganglion cells between the muscle layers of a gastroenterogenous cyst. Hemalum and eosin; reduced ⅙ from mag. × 400.

present in all cysts (Fig. 10). Gastric mucosa and glands with chief and parietal cells formed the predominant lining of three cysts (Fig. 8). One of the three had a small area of squamous epithelium and two small foci of pseudostratified ciliated columnar epithelium (Fig. 11). In some places the lining epithelium consisted of a single layer of columnar mucus-secreting cells (Fig. 9) supported by a thin substantia

Fig. 11.—Gastroenterogenous cyst with an area of mucosa composed of ciliated, pseudostratified, columnar epithelium. Hemalum and eosin; reduced about ⅙ from mag. × 275.



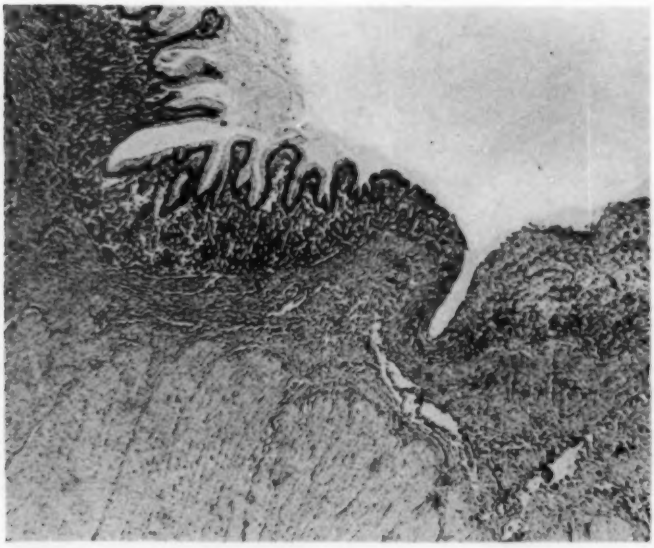


Fig. 12.—Gastroenterogenous cyst with an area of superficial ulceration considered indicative of peptic digestion. Hemalum and eosin; reduced slightly from mag. × 60.

propria. No duodenal or jejunal glands were identified. The fourth cyst, from the patient 5 years of age, had no gastric mucosa and was lined primarily by a zone of fibrinoid material over maturing granulation tissue. Where epithelium remained, it consisted of a single layer of columnar cells. Small chronic ulcers were present in the other cysts, but they did not extend into the muscularis (Fig. 12). Foci of lime salts were present in the ulcerated areas of two cysts.

Comment.—Although gastroenterogenous cysts are less frequent than bronchogenous and pericardial cysts, they have attracted more attention, especially with respect to their formal genesis. Mixter and Clifford [7] and Poncher and Milles [37] thought that these cysts develop by sequestration of diverticula or epithelial nodules from the foregut during early development. Black and Benjamin [43] expressed the view that they are intrathoracic vestiges of the ductus omphalomesentericus. The theory that they are duplications of the intestinal tract was supported by Evans,[23] Ladd and Scott,[35] and Keith.[44] These authors considered that such cysts arise from persistent vacuoles that normally appear at the time of canalization of the primitive intestinal tract, but that fail to coalesce in a longitudinal plane. Bremer [27] expressed the opinion that gastroenterogenous cysts may be derived from developmental diverticula or may represent short segments of duplication. These theories, however, do not consider the almost constant association of these cysts with malformation of the cervical and thoracic vertebrae, as noted by Olken [32]; Veeneklaas [41]; Kuipers and Wieberdink,[30] and Fallon, Gordon, and Lendrum.[15] The last-mentioned authors and Veeneklaas [41] noted that the entodermal tube and the notochord are closely approximated in early embryonic development. They postulated that the two become adherent and that as they separate part of the entodermal tube is drawn out as a traction diverticulum. This becomes cut off from the foregut, and a gastroenterogenous cyst results. This theory explains the very frequent close relationship of the cysts to the vertebrae and the frequency of vertebral malformations. The

observations on the four cysts of this series support this concept. The occasional association with intra-abdominal enteric cysts or duplications is not adequately explained.

The predominance of male infants and children in reported collections ‡ of gastroenterogenous cysts is supported by the four cases reported here. The occurrence of symptoms in all cases is also in agreement with previous reports and is in contrast to the absence of symptoms for most bronchogenous cysts. The symptoms are attributed usually to pressure on thoracic structures, but rupture into bronchi with massive hemoptysis and death have been reported.§ This is due to perforation of peptic ulcers. Superficial necrosis with ulceration, as observed here, is a common finding and is considered peptic in nature. Calcification also is frequent, and Steele and Schmitz [48] reported ossification in a cyst from a 15-year-old girl.

Valle and White [47] divided gastrogenous cysts into two types, acid-secreting, functionally active cysts and those in which the mucosa is without functional activity. Many cysts, originally of the functional type, conceivably may lose functional activity with destruction of the secretive areas of mucosa. Positive tests for rennin, pepsin, chlorides, and free hydrochloric acid on the contents of some of the cysts have been reported.‖

The diagnostic feature of cysts of this type is the thick muscular wall which duplicates that of stomach or small intestine. The mucosa is variable, as might be expected, since the cysts arise from the primitive foregut. Usually there is some combination of gastric, enteric, squamous, ciliated pseudostratified, or columnar mucus-secreting mucosa. The presence of ciliated epithelium does not justify the conclusion that the mucosa is respiratory. Gastric glands are most frequent, but esophageal, duodenal, or small intestinal glands may be found. It is impossible to divide this group of cysts categorically into gastrogenous, duodenal, and enteric forms, and therefore the term "gastroenterogenous" is preferred for descriptive reasons. The term "foregut cyst" is of some value with respect to formal genesis, but would include the esophageal cysts which are structurally different.

‡ References 32, 34, and 35.

§ References 45 through 47.

‖ References 33 and 35.

PERICARDIAL CELOMIC CYSTS

Pickardt [49] is credited with the first successful removal of a pericardial celomic cyst from the mediastinum, in 1934. He termed it a "pleurodiaphragmatic cyst." Similar cysts, but not their true nature, were recognized in the nineteenth century.[50] Lambert,[51] in 1940, reported two symptomless cysts that were discovered on routine examination and removed successfully. He stressed the developmental origin from the pericardial celom and suggested the term "pericardial celomic cyst" as accurately defining their true nature. The term "spring-water cyst" is attributed indirectly to Churchill by Greenfield and co-workers.[52] Cysts of similar type have been designated simple hydrocele of the mediastinum,[53] simple cyst of the mediastinum,[53] mesothelial cyst,¶ pericardial celomic cyst of Lambert,[54] and cardiophrenic angle cyst.# They have been confused with cystic lymphangiomas.*

Blades' [2] series of mediastinal tumors included 10 pericardial cysts as compared with 23 bronchogenous cysts. Only 2 of the 17 cysts recorded by Sabiston and Scott [4] were of the pericardial type. Lillie, McDonald, and Clagett,[50] in 1950, collected 25 examples from the literature and reported 12 cases. Forsee and Blake (1952)[61] accepted reports of 62 cysts that had been removed surgically.

Clinical Observations. — In the present series there were eight pericardial celomic cysts which had been removed successfully. The ages of the patients ranged from 32 to 49 years, and there was an equal number of cysts for each sex (Table 2). Symptoms were present in four patients. The other four cysts were incidental findings on roent-

¶ References 54 and 55.

References 56 and 57.

* References 58 through 60.

genographic survey. One cyst was followed radiographically for four years and showed very gradual but progressive increase in size. Five cysts were located in the right anterior costophrenic angle, two in the left costophrenic angle, and one in a supracardiac position partially overlying the pericardial sac. Fibrous pedicles were described for five cysts with attachments to the parietal pericardium. Two cysts were attached also to the diaphragm. The remaining two appeared to be separated completely from the pericardium.

Structure.—All cysts contained a clear serous transudate. Seven were unilocular and one multilocular. The walls were very thin and the inner surfaces smooth and glistening. They varied from 6 to 30 cm. in greatest diameter and were lined by a single layer of flat mesothelial cells (Fig. 13). In several cysts the lining cells were hypertrophied and cuboidal. The mesothelium was supported by fibrous tissue with attached adipose tissue. Inflammation was absent or minimal.

Fig. 13.—Pericardial celomic cyst lined by a single layer of flat mesothelial cells. Hemalum and eosin; reduced about ⅓ from mag. × 640.

Comment.—The evidence indicates that these mesothelial cysts are developmental and that the formal genesis is related to the pericardial celom, as suggested originally by Lambert.[51] Drash and Hyer[54] and Cooper, Archer, and Mapp[55] stated that Kindred † postulated the origin as aberrant growths from the mesothelium of the pleural cavity, when it invades the mesenchyme of the body wall. However, pericardial origin was supported by the investigations of Lillie, McDonald, and Clagett[50] and by clinical observations. These authors pointed out that the primitive pericardial cavity forms by the fusion of celomic spaces on each side of the embryo. During the process, dorsal and ventral parietal recesses are formed. The dorsal recesses communicate with the pleuroperitoneal celom. The ventral recesses end blindly at the septum transversum. They concluded that the persistence of segments of the ventral parietal recesses would account for most pericardial celomic cysts and diverticula. This theory explains adequately the location of the cysts in the cardiophrenic angles and/or the diaphragm. There is no explanation for the greater frequency on the right side. Cysts of suprapericardial location may be derived also from the ventral recesses, but have been left cephalad when the septum transversum descends. Certain reported mesothelial cysts ‡ may be related

† Kindred, J. E.: Cited as a personal communication (references 54 and 55).

‡ References 56, 62, and 63.

to persistent segments of the dorsal recesses, or they may be of pleural origin, as has been suggested by Kindred § and others.‖

The clinical observations and the histologic features of the pericardial cysts of this series are in agreement with those of others. These cysts give little difficulty in diagnosis and can be differentiated from cystic lymphangiomas in that they are usually unilocular and do not possess prominent bundles of smooth muscle fibers in the walls or aggregates of lymphocytes.

THYMOGENOUS CYSTS

Multiple small cysts of the thymus are observed frequently in necropsy material, but there are very few reports in the literature of large thymogenous cysts. Neither Laipply [6] nor Blades [2] mentioned such cysts. Of 17 mediastinal cysts, Sabiston and Scott [4] referred to 4 which were located in the anterior mediastinum and showed no characteristic structure. One or more of these cysts may have been thymogenous. The first thymic cysts that were resected successfully from the anterior mediastinum were reported by Bradford, Mahon, and Grow [65] and by Smart.[66] The cyst resected by Bradford and co-workers [65] was from a 22-year-old man and was multilocular. The one reported by Smart,[66] from a 53-year-old woman, consisted of a large cyst with smaller compressed cysts in the wall. Fridjohn [67] described a large cyst in the thymus of a one-day-old infant. Thymic cysts have been resected from the neck.¶ By 1954, Krech, Storey, and Umiker [70] found reports of eight cysts that had been resected from the mediastinum. They reported two additional examples and referred to two more in an addendum. All four were from adults. The authors recognized cysts of three types: congenital, inflammatory, and neoplastic. They presented evidence that the cysts reported by them were of the congenital type.

§ Kindred, J. E.: Cited as a personal communication (references 54 and 55).

‖ References 62 through 64.

¶ References 68 and 69.

Clinical Observations.—In the present series, two cysts of the anterior mediastinum were considered to be thymogenous. They were resected from women whose ages were 45 and 50 years. The first cyst was detected in a routine roentgenogram, and its presence had caused no symptoms. It was not visible on a roentgenogram taken three years previously. The second cyst produced symptoms due to pressure on the trachea.

Structure.—The first specimen was multilocular, with one large and several small cystic spaces. It measured 7 cm. in diameter and contained a thin, dark straw-colored fluid. The cyst was lined by cuboidal or flattened epithelium, but in some areas there was no epithelium and the inner surface consisted of a layer of old blood with hemosiderin-containing macrophages (Fig. 14). The wall was formed by thick, hyaline, fibrous tissue with whorled areas. Incorporated in the fibrous wall were foci of

Fig. 14.—Thymogenous cyst. Part of the lining is visible at the upper right and consists of a single layer of flattened cells. It is disrupted by old and recent hemorrhage. Thick hyaline connective tissue with small collections of lymphocytes comprises the wall. Hemalum and eosin; reduced about ¼ from mag. × 100.

thymic syncytial cells and lymphocytes (Fig. 15). Several areas suggested Hassall's corpuscles. Outside the fibrous wall were compressed lobules of thymic tissue and fibroadipose tissue.

The second cyst measured 18 cm. in greatest diameter. It was unilocular and contained a thick brownish red fluid due to old and recent hemorrhage. Where the lining epithelium was visible, it consisted of pavement cells. In most areas, however, it was replaced by granulation tissue with lipidic and hemo-

Fig. 15.—Compressed thymic tissue, lymphocytes, and parenchymatous cells, in the wall of the cyst shown in Figure 14. Hemalum and eosin; reduced about ¼ from mag. × 125.

siderin-containing macrophages. The wall consisted of thick fibrous tissue with islands of thymic tissue. Thymic tissue was adherent also to the outer wall of the cyst.

Comment.—The structural features of the two cysts were similar to those of previous reports.# Krech, Storey, and Umiker [70] concluded that cysts of this type were congenital and arose from persistent elements of the thymic or thymopharyngeal duct, which had become distended by fluid and blood.

Thymogenous cysts of this type must be differentiated from lymphangiomas that arise in or near the thymus. It is also possible that cystic bigerminal teratomas could be confused with thymic cysts, when the lining epithelium has been destroyed. The two cysts that are reported here are presumed to be congenital in origin and to have arisen from persistent elements of the thymic duct.

MENINGOCELE

Intrathoracic meningoceles are not true cysts and do not arise within the true mediastinum but, like some neurogenous tumors, they may extend into the mediastinum. From a diagnostic point of view, it is practical to consider them with mediastinal cysts. The first example was reported by Pohl,[72] in 1933. The patient had von Recklinghausen's neurofibromatosis also, and the clinical diagnosis was probable neurofibroma. Upon exploration, a meningocele was found to connect with the spinal subarachnoid space by a 2 cm. lateral defect at the level of the fourth dorsal vertebrae. The second lateral meningocele in association with neurofibromatosis, confirmed at necropsy, was reported in 1938 by Schüller and Uiberall.[73] Ameuille, Wilmoth, and Kudelski [74] described an intrathoracic meningocele without neurofibromatosis. The first intrathoracic meningocele that was resected successfully was reported by Welch, Ettinger, and Hecht,[75] in 1948. The patient also had neurofibromatosis. Byron, Alling, and Samson [76] recorded three cases of proved intrathoracic meningocele. In each instance the preoperative diagnosis was neurofibroma. Case 1 of their group is the example reported here.

Clinical Observations.—The patient was a 36-year-old woman. There was a roughly spherical, posterior-superior mediastinal and right intrathoracic mass, which was known to have been present for over five years. The clinical diagnosis was neurofibroma. The mediastinum and right thoracic cavity were

References 66, 70, and 71.

explored, and a cyst was found which communicated with the spinal subarachnoid space by a lateral defect in the spinal column. This defect measured approximately 3 by 2 cm. The cyst was resected and the defect closed. Recovery from the procedure was excellent. There were no neurofibromas but there were café-au-lait spots on the trunk.

Structure.—The cyst measured 12 cm. in its greatest diameter and contained approximately 2500 ml. of clear watery fluid. The inner surface was smooth and glistening.

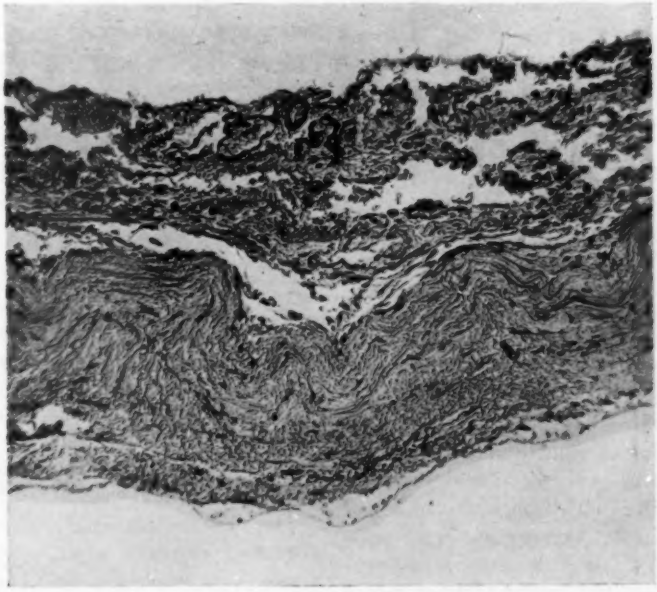


Fig. 16.—Meningocele. The wall of the sac consists of an inner, loosely arranged, arachnoid mater and an outer, hyaline, fibrous dura mater. Hemalum and eosin; reduced ⅓ from mag. × 150.

The wall was formed by two distinct components which represented the dura mater and arachnoidea spinalis (Fig. 16). The dural component consisted of a thick layer of relatively acellular, hyaline, fibrous tissue. In some areas, small nerve trunks and ganglia were attached to, or incorporated in, the outer portion of the wall. The arachnoidal component formed the lining of the cyst and was adherent to the dura in areas. Elsewhere it was separated from the dura by longitudinal clefts lined by arachnoidal mesothelium. The arachnoid consisted of a very vascular and loosely arranged web of connective tissue with small spaces lined by arachnoidal cells, and villi covered by similar cells. There was no calcification or inflammation.

Comment.—The frequent coexistence of von Recklinghausen's neurofibromatosis and mediastinal or intrathoracic meningocele cannot be dismissed as coincidence. It indicates an intrinsic causal factor common to both lesions. The case presented did not have obvious neurofibromatosis, but café-au-lait spots suggested that it may have been present in a latent or minor form. Of the 10 intrathoracic meningoceles that were reviewed,* 7 were associated with obvious neurofibromatosis. Whenever a well-defined posterior mediastinal or intrathoracic lesion is observed in a patient with neurofibromatosis, the possibility of a meningocele must

* References 72 through 79.

be considered. A preoperative diagnosis is possible by the use of myelography, as has been suggested.† At least five meningoceles have been resected successfully.‡ As long as the possibility is considered, no difficulty is encountered in respect to either clinical or histologic diagnosis.

The mechanism of development of mediastinal meningoceles probably involves the extension of dura and subarachnoid through a spinal nerve foramen. This process becomes dilated and finally cystic. As a result of pressure, there is enlargement of the foramen and the formation of a large lateral defect.

SUMMARY

Thirty-six primary developmental cysts of the mediastinum, other than cystic teratomas, have been presented in this report. They comprised 27% of 133 primary mediastinal cysts and neoplasms from which material was secured by a definitive surgical procedure or for biopsy.

With 17 examples, the tracheobronchogenous cyst was the commonest variety. These were excised from patients whose ages ranged from 17 to 56 years. Histologically, these cysts reproduced the structure of the trachea or bronchus. The walls contained bundles of smooth muscle, and all were lined by ciliated, pseudostratified, columnar epithelium. Bronchial glands and cartilaginous plates were present in the walls of most cysts. Cysts of the paratracheal subgroup were located on the right side and are considered to represent cystic remnants of a lateral bronchus.

Pericardial celomic cysts were next in frequency. Eight of these were resected, four from each sex. The ages of the patients ranged from 32 to 49 years. Seven cysts were located in the anterior costophrenic angles, five on the right side. All contained a clear serous fluid. The walls consisted of a thin layer of fibrous connective tissue, which was lined by a single layer of mesothelial cells. Such cysts are thought to be derived from remnants of the ventral parietal recesses of the primitive pericardial celom.

There were four gastroenterogenous cysts. Three patients were male infants, and the fourth was a boy 5 years of age. In each case there were associated congenital malformations of thoracic vertebrae. These cysts were attached also to the spinal column, indicating a formal genesis involving the spinal column or notochord. It can be presumed that a traction diverticulum at a point of adherence subsequently became separated as a cyst. The characteristic thick muscularis was divisible into either two or three layers. The lining mucosa was predominantly gastric in type, and there were multiple superficial peptic ulcers.

Three esophageal cysts were examined. Two resembled well-developed esophagus, and the wall contained a mixture of smooth and striated muscle. The lining was moist squamous epithelium, and there were esophageal glands. The third cyst resembled fetal esophagus and was lined by ciliated pseudostratified epithelium. The cysts that resembled adult esophagus were considered to represent sequestrated congenital diverticula. The ciliated epithelial cyst was thought to have developed from persistent vacuoles that failed to coalesce longitudinally, so that the normal continuity of the lumen was not established.

One cyst, designated as tracheoesophageal, contained elements which resembled both trachea and esophagus. This cyst either had its inception during the very early formation of the tracheal groove, or it represented a cystic remnant of a tracheoesophageal fistula.

Two anterior-superior mediastinal cysts in adult women had very thick fibrous walls, with islands of thymic tissue in relation to the outer surface. Both were lined in part by a layer of flattened cells. They were thought to have origin in persistent elements of the thymopharyngeal duct.

One mediastinal and intrathoracic meningocele was resected successfully from a 36-year-old woman. These cysts are usually associated with von Recklinghausen's neurofibromatosis. They are thought to represent

† References 73 and 77.

‡ References 75 through 79.

cystic distention of dura mater and arachnoidea spinalis which have extended through a spinal foramen.

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Thromboembolism, Pulmonary Arteriosclerosis, and Fatty Meals

An Experimental Study of the Effect of Intermittent Fatty Meals on Thromboembolic-Induced Pulmonary Arteriosclerosis in Rabbits

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Most of the serious complications of arteriosclerosis result from arterial occlusion, and the occluding material is usually a combination of fat, fibrous tissue, and blood clot. Much of the investigation of arteriosclerosis is centered around the sequence in which these three appear, their relation to each other, and factors that influence any of the three.

Many, but not all, authorities believe that in systemic vessels deposition of fat is the initiating event, that fibrosis follows, and that thrombosis is a late complication. However, in small pulmonary vessels considerable experimental,* pathological,† and clinical ‡ evidence has been presented indicating that the initiating event is often thrombosis (due to embolization), followed by fibrosis and, in man, fat deposition.[6] In experimental animals fat has seldom been found in pulmonary arterial lesions induced by embolization.§

Rabbits have been used exclusively in all of the reported experiments in which pulmonary arteriosclerosis has been produced by repeated injections of blood clot. The natural diet of the rabbit and the diets ordinarily given to domesticated rabbits contain very little fat, and this fact may explain why fibrous intimal lesions of the pulmonary arteries of rabbits on ordinary diets contain little or no fat. A method of adding fat to lesions produced by thromboemboli in pulmonary arteries of animals would provide an opportunity to explore further the relation of fat, fibrosis, and thrombosis. Data obtained from such experiments should be useful in investigating not only the special types of arteriosclerosis known to result from thrombosis but also factors that influence fat, fibrosis, and thrombosis in any form of arteriosclerosis.

In a preliminary experiment stainable lipid was demonstrated in pulmonary arterial thrombi in a rabbit that had received repeated weekly intravenous injections of blood clots and several feedings of 40% cream by gastric intubation during a 24-hour period prior to killing. Having thus obtained evidence suggesting that fat could be introduced into pulmonary arterial thrombi, we designed a series of experiments to test the effect of intermittent fatty meals on pulmonary arterial lesions in rabbits induced by thromboemboli. The purpose of this report is to present the results of these experiments.

MATERIAL AND METHODS

Healthy young adult New Zealand white rabbits of both sexes were used as experimental animals. They were kept in air-conditioned animal rooms, fed 100 gm. of Purina rabbit pellets daily, and offered water as desired.

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* References 1 and 2.

† References 3 and 4.

‡ References 4 and 5.

§ References 1, 2, and 7.

Whole blood for preparation of a clot suspension was obtained by cardiac puncture from other healthy rabbits. This blood was beaten rapidly with a glass or metal rod. The resultant clot consisted largely of fibrin, but there were some enmeshed erythrocytes. The clot was separated from the fluid portion of the blood and placed in a Waring Blendor. Enough isotonic saline was added to the clot to produce a mixture with the same volume as that of the whole blood from which the clot had been obtained. The Waring Blendor was operated at high speed for 5 to 10 minutes, breaking the clot into fragments that would pass readily through an 18-gauge needle but not readily through a 20-gauge needle. There was some tendency for the fragments of clot to settle, and in order to maintain a uniform suspension the mixture was stirred with the Waring Blendor for one to two minutes prior to each injection. Injections were given weekly into the ear veins through 18-gauge needles. As a precaution against unequal dosage, the sequence in which the rabbits receiving clot were injected was changed each week (those receiving the first portion one week would receive the last portion the next week).

The fatty meals used in this experiment consisted of either commercial butter or oleomargarine. The oleomargarine was derived from soybean and cottonseed oil and contained no animal fat. The butter and the oleomargarine were melted separately (the melting point of each was approximately 29 C), and one or the other was given at a temperature of approximately 40 C to designated rabbits through a rubber tube that had been introduced transorally into the stomach. The quantity of each fat feeding was 25 ml.

The following groups of rabbits were established:

Group I ("butter-clot" group).—Fifteen rabbits were given 5 to 10 ml. of the clot-suspension intravenously once weekly for six weeks. Immediately following each intravenous injection a rubber tube was inserted into the stomach transorally, and 25 ml. of melted butter was introduced. Ten of these rabbits were given a seventh intragastric feeding of butter (but no clot) 8 to 13 days after the last injection of blood clot. Rabbits were killed with intravenous formalin at the times designated in Table 1.

Group II ("oleomargarine-clot" group).—Twelve rabbits were subjected to the same regimen as the "butter-clot" group, except that oleomargarine was used instead of butter.

Group III ("water-clot" group).—Fifteen rabbits were subjected to the same regimen as the preceding groups, except that warm (40 C) water was introduced through the gastric tube instead of butter or oleomargarine.

Group IV ("butter-saline" group).— Four rabbits were subjected to the same regimen as the "butter-clot" group, except that sterile isotonic saline was injected intravenously instead of the clot-suspension.

Group V ("oleomargarine-saline" group).—Four rabbits were given oleomargarine through the gastric tube, according to the same schedule used for the "oleomargarine-clot" group, and saline intravenously.

Group VI ("normal-control" group).—Twenty-five rabbits that had not been subjected to any experimental procedures were examined.

Complete autopsies were performed on all animals, and selected portions of tissue, including the entire thoracic contents, were fixed in 10% formalin for at least five days. The hearts were then separated from the lungs and pericardium by sectioning the great veins at the pericardium and cutting across the aorta and pulmonary artery at the superior borders of their valve cusps. The atria were removed by cutting along the atrioventricular groove. The right ventricle was then separated from the interventricular septum. The weight of the right ventricle, without the interventricular system, divided by the weight of the left ventricle and entire interventricular system will be referred to hereinafter as the "right ventricular-left ventricular ratio."

One block of tissue was taken from each upper and each lower lobe of the lungs for paraffin sections and from each lower lobe for frozen sections. Sections from all paraffin blocks were stained with aldehyde fuchsin-Van Gieson-iron hematoxylin, and selected tissues were stained with hematoxylin and eosin. The frozen sections were stained with oil red O. Lesions found in small pulmonary arteries were classified as either thrombi or fibrous intimal thickening and graded according to a system previously used by us in a study of human material.[6] A similar system was used for grading the amount of fat present. Frequency and severity were graded separately. Only the sections stained by the method employing aldehyde fuchsin-Van Gieson-iron hematoxylin were used for grading, and all of the pulmonary tissue from each animal was considered as a unit. A grade of "1" for frequency indicates that one to three lesions of the type designated were encountered. A grade of "2" indicates four to six, and a grade of "3" indicates more than six. As applied to fat, it indicated small, medium, or large amounts. Severity of fibrous intimal thickening was graded as follows: Grade 1 indicates a lesion that is less thick than the media, Grade 2 indicates a lesion as thick as the media, and Grade 3 one that is thicker than the media. The grading for severity was not applied to thrombi or fat. In order to simplify charting of results, grades for frequency and severity were combined and a single figure was obtained as follows: If either frequency or severity had been graded as "1," a grade of "1" was assigned for combined frequency and severity. If both frequency

TABLE 1.—*Comparative Pathologic Data from Experimental Groups Receiving Clots* *

	Clot-Butter					Clot-Oleomargarine					Clot-Water				
	No. of Rabbit	Clot-Suspension, Ml.	Thrombi	Fibrous Intimal Thickening	Fat in Lesions	No. of Rabbit	Clot-Suspension, Ml.	Thrombi	Fibrous Intimal Thickening	Fat in Lesions	No. of Rabbit	Clot-Suspension, Ml.	Thrombi	Fibrous Intimal Thickening	Fat in Lesions
I †	1	60	0	1	1	16	60	2	2	1	28	50	0	1	0
	2	60	3	3	2	17	60	3	2	0	29	60	0	1	1
	3	60	0	2	1	18	60	2	2	0					
	4	60	2	2	0	19	60	1	1	1					
II ‡	5	60	2	2	0	20	60	2	2	0	30	60	1	2	0
	6	60	1	2	0	21	60	1	2	0	31	60	1	1	0
	7	60	2	3	0	22	60	1	2	0	32	60	0	1	0
	8	60	1	1	0	23	60	2	1	0	33	60	1	2	0
	9	60	1	2	0						34	60	0	1	0
	10	60	1	3	1						35	60	1	2	0
III §	11	75	0	3	0	24	80	2	1	0	36	75	2	1	1
	12	50	3	2	3	25	75	3	2	3	37	80	0	2	0
	13	50	3	2	1	26	70	3	2	1	38	80	2	1	1
	14	50	3	3	0	27	60	0	2	0	39	75	1	1	2
	15	65	3	2	1						40	45	3	3	1
											41	75	2	3	0
											42	45	2	1	1

* System for grading pathologic lesions is given in text. Grades 0 to 3 are based on frequency and severity.

† Animals killed 8 days after last i.v. injection and 1 day after last gastric feeding.

‡ Animals killed 13 days after last i.v. injection and 2 days after last gastric feeding.

§ Animals killed 6 days after last i.v. injection and 6 days after last gastric feeding.

TABLE 2.—*Number and Percentage of Rabbits in Various Groups with Moderate or Severe Fibrous Intimal Thickening of Small Pulmonary Arteries*

Combined Grade of 2 or 3 for Frequency and Severity *

	Rabbits, No.		Grade 2 or More, No.		Grade 2 or More, %	
Clot-butter	15	27	13	22	87	81
Clot-oleomargarine	12		9		75	
Clot-water	15		6		40	
Saline-butter	4		0		0	
Saline-oleomargarine	4		0		0	
Untreated	25		0		0	
Totals						
With clot	42		28		67	
Without clot	33		0		0	

* Grading system is given in text.

and severity were graded as "2," a grade of "2" was assigned. If either frequency or severity was graded as "2," and the other graded as "3," or if both were graded as "3," a combined grade of "3" was assigned.

RESULTS

Gross Observations.—No significant gross abnormalities of the heart or lungs were noted, except that the right ventricular-left ventricular ratio was significantly larger in animals subjected to embolization than in the normal controls (p—0.01). Moreover, the right ventricular-left ventricular ratio was significantly larger in the rabbits subjected to embolization and fatty meals than in the rabbits subjected only to embolization (p—0.05). See Table 3.

TABLE 3.—*Average Ventricular Weights in Various Groups*

	RV *	LV †	RV/LV, %
Clot-butter	1397	4160	34
Clot-oleomargarine	1568	4700	34
Clot-water	1520	4836	31
Totals for all clot groups	1495	4563	33
Untreated and saline i.v. groups	1551	5343	29

* Right ventricle minus septum, in milligrams.
† Left ventricle including entire septum, in milligrams.

Microscopic Observations on the Lungs.—Vascular lesions in rabbits subjected to embolization were confined to small pulmonary arteries (none were noted in arteries with an external diameter greater than 300μ) and were qualitatively, but not quantitatively, similar in all groups. The arterial lesions were similar to those that have been described by Harrison,[1] Wartman,[7] and other investigators, and hence they will be described only briefly in this paper. In a few rabbits that died immediately after the last injection, fresh fibrin thrombi were found in otherwise normal vessels (Fig. 1). Many other rabbits had similar thrombi that appeared to have been invaded by large numbers of granulocytes, lymphocytes, and monocytes, and similar cells were sometimes present in the adjacent arterial wall (Fig. 2). Such thrombi, if retracted from the vessel wall, were partially covered with endothelial cells, and there was invasion by plump fibroblasts. Occasionally the internal elastica of the containing vessel was partially destroyed (Figs. 3 and 8), but in most it was intact. In general, beginning organization of thrombi was associated with retraction from one side of the vessel wall. Other thrombi showed more advanced organization and further retraction to one side of the vessel wall, although they still protruded into the lumen in an irregular fashion. Some such thrombi still contained fibrin (Figs. 4 and 6), and some contained dense, hyalinized collagen (Fig. 5). Typical recanalized, partially organized thrombi (Fig. 6) were common.

All of the lesions thus far described are clearly recognizable thrombi. In addition to these, other lesions were seen that cannot be classified quite as readily. However, the dividing line between these and certain of the clearly recognizable thrombi is not a sharp one. The second type of lesion consisted of some form of fibrous tissue forming an eccentric (Fig. 8) or an approximately concentric plaque along the inner surface of an artery (Figs. 7 and 9). For the lack of better terminology, this type of lesion is referred to in this report simply as fibrous

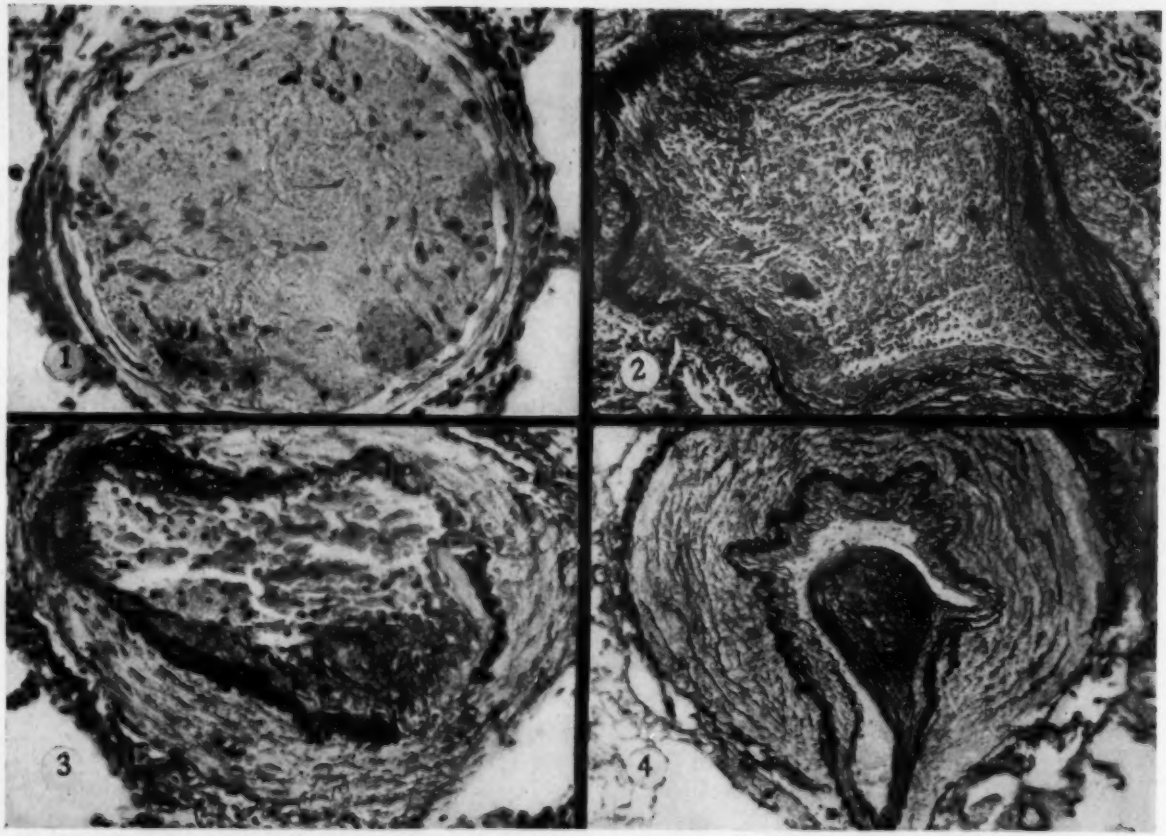

Fig. 1.—Rabbit 12 (clot-butter group). A recent fibrin thrombus occluding a small muscular artery. The lumen of the vessel appears distended. Aldehyde fuchsin-Van Gieson; × 230.

Fig. 2.—Rabbit 40 (clot-water group). An occluding arterial thrombus undergoing organization. Fibroblasts are present throughout the thrombus. A brisk acute inflammatory cell infiltrate extends through the arterial muscular wall and into the adventitia. Aldehyde fuchsin-Van Gieson; × 95.

Fig. 3.—Rabbit 12 (clot-butter group). An eccentric fibrous intimal lesion. The internal elastic lamina is interrupted beneath the lesion. Aldehyde fuchsin-Van Gieson; × 230.

Fig. 4.—Rabbit 10 (clot-butter group). An organizing thrombus protruding into the arterial lumen. In the center of the lesion a small amount of fibrin remains. Aldehyde fuchsin-Van Gieson; × 95.

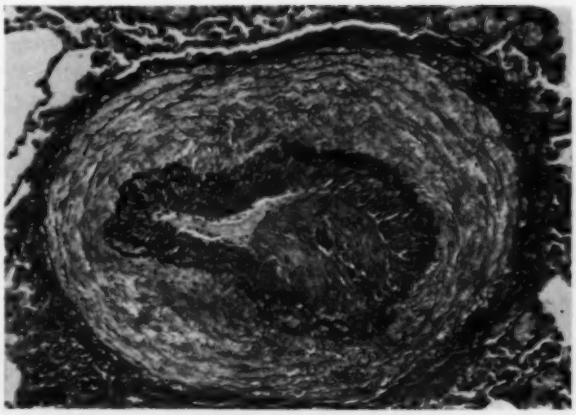

Fig. 5.—Rabbit 13 (clot-butter group). An organized, collagenized thrombus partially occludes the vessel. The intima is slightly thickened in its entire circumference. Aldehyde fuchsin-Van Gieson; × 95.

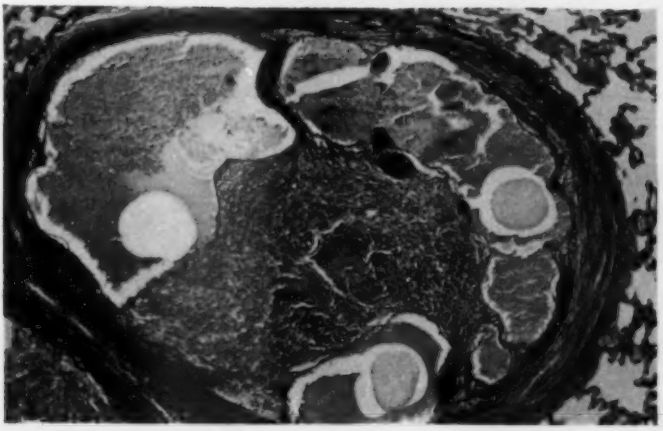

Fig. 6.—Rabbit 39 (clot-water group). An organizing, recanalized thrombus in a large pulmonary artery. Fibrin remains in the center of the lesion. Aldehyde fuchsin - Van Gieson; reduced from mag. × 80.

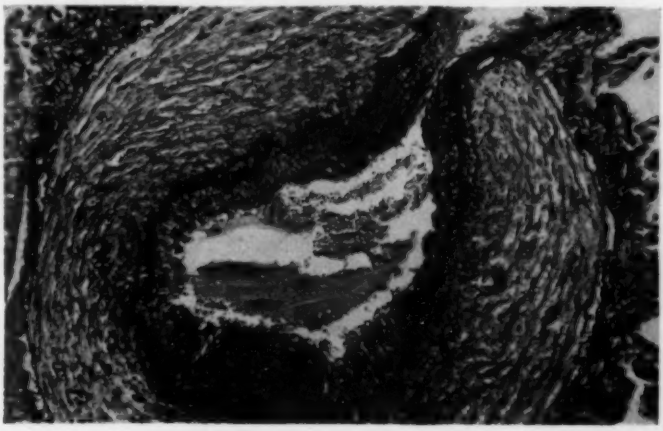

Fig. 7.—Rabbit 41 (clot-water group). The fibrous intimal thickening is almost concentric, stopping rather abruptly at the origin of an arteriole. Aldehyde fuchsin-Van Gieson; reduced from mag. × 190.

intimal thickening. In most instances the fibrous tissue was loose and cellular, but occasionally it was hyalinized or contained elastic fibers. The internal elastica was usually intact beneath such lesions, but in some instances it was partially or completely destroyed and fibrous tissue had extended into the media (Figs. 4 and 8).

Fat was demonstrated in all types of lesions from all groups, but it was not a constant or even a common feature. It usually took the form of finely dispersed droplets, but in some instances large globules were present (Figs. 10, 11, and 12).

and severity of lesions between the butter-clot group and the oleomargarine-clot group is not statistically significant, but the difference between the water-clot group and both groups receiving lipid is highly significant ($p<0.01$).

Fat was demonstrated in pulmonary arterial lesions of 47% of the rabbits in the butter-clot group, 33% in the oleomargarine-clot group, and 40% in the water-clot group. None of the differences among these groups is statistically significant. In all groups, fat was encountered in only a small percentage of the lesions.

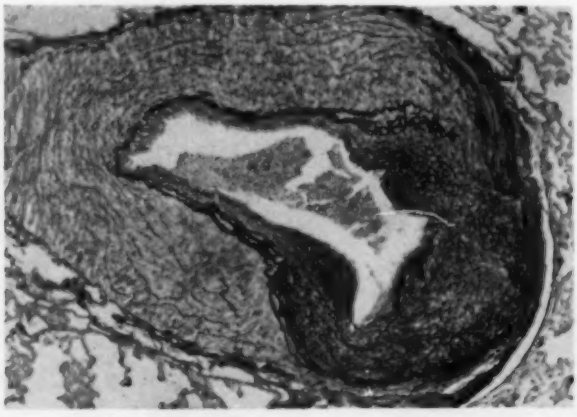

Fig. 8.—Rabbit 10 (clot-butter group). An eccentric fibrous intimal lesion in a muscular artery. The media beneath the lesion appears thinned and the internal elastica is broken. There has been marked elastic tissue proliferation within the fibrous tissue. Aldehyde fuchsin-Van Gieson; × 75.

The frequency and severity (combined grade) of thrombi, fibrous intimal thickening, and fat in each rabbit are indicated in Table 1.

Grade 1 fibrous intimal thickening was not uncommonly encountered in control groups (untreated and treated with saline injections), but Grade 2 fibrous intimal thickening was found only in animals that had received intravenous injections of blood clot. Grade 2 or 3 fibrous intimal thickening was found in 87% of the butter-clot group, 75% of the oleomargarine-clot group, and in 40% of the water-clot group. The difference in frequency

COMMENT

Presence of Fat in Lesions of Rabbits on Regular "Pellet" Diet Only.—The discovery of fat in the pulmonary arterial lesions of 40% of the rabbits in the water-clot group was quite unexpected. Previous investigators have invariably reported the rarity or absence of fat in similar lesions of rabbits subjected to repeated embolization with blood clots.‖ The clot-suspension used in our experiments probably contained more erythrocytes than the washed fibrin clot used by Harrison (who

‖ References 1, 2, and 7.

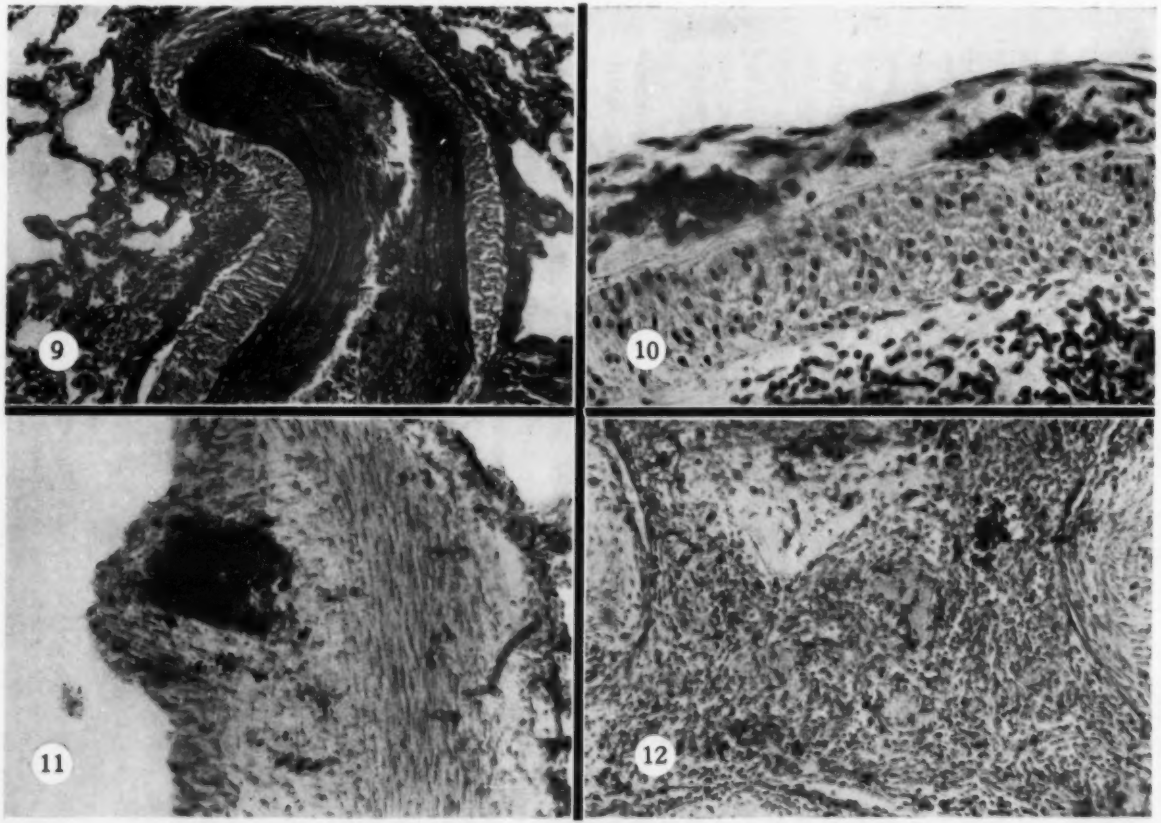

Fig. 9.—Rabbit 40 (clot-water group). An almost concentric intimal proliferation of fibrous tissue. The intimal surface is ragged, and fibroblasts remain in the lesion. Aldehyde fuchsin-Van Gieson; × 95.

Fig. 10.—Rabbit 1 (clot-butter group). Globules of fat lying within the thickened intima. The fat appears as black clumps. Oil red O stain; × 230.

Fig. 11.—Rabbit 39 (clot-water group). An aggregation of fat droplets within a fibrous intimal lesion. Oil red O stain; × 95.

Fig. 12.—Rabbit 12 (clot-butter group). Scattered clumps of lipid (appearing black) within an organizing, occluding thrombus. Oil red O stain; × 95.

found fat in a single lesion in only 1 of 10 rabbits), but less than the whole blood clot used by Wartman and co-workers (who found no fat in the arterial lesions of 14 rabbits). However, the amount of clot (as determined by the quantity of whole blood from which it was obtained) used in our experiments for each injection was 10 times greater than that used by Wartman and co-workers [7] and 5 times greater than that used by Harrison.[1] Since the individual doses were larger, the total amount of fat obtained from the lipid capsule of the erythrocytes may have been greater than that obtained by other investigators, and it is at least possible that this accounts for the difference in results.

The diet of the water-clot rabbits consisted of Purina pellets, which contain only a small amount of fat (approximately 2%, according to the manufacturer). Similar pellets were used by Wartman and co-workers.[7] The amount of fat in commercial preparations such as Purina pellets may vary, but it seems unlikely that this variation is enough to account for the difference in results.

Regardless of the cause, the presence of fat in the fibrous intimal lesions of the rabbits increases their similarity to the fibrous intimal lesions seen in the pulmonary arteries of humans.[6]

Possible Mechanisms by Which Intermittent Fatty Meals Increase the Severity of Pulmonary Arterial Lesions.—The difference in frequency and severity of arteriosclerotic lesions in the rabbits that received supplementary fat as compared with those that did not receive supplementary fat is statistically highly significant. If more fat had been found in the lesions from the rabbits that had received fat than in the others, the most obvious explanation for the difference in incidence and severity would have been that the presence of fat stimulated fibrosis. However, no significant differences were detected in the amount of fat present in the arteriosclerotic lesions in the various groups. Nonetheless, more fat than that found at the time of autopsy could have been present at some time during life in lesions of the rabbits that had been given supplementary fats, and this could account for the difference in incidence and severity.

Another possible explanation is that the fatty meals could have resulted in temporary alterations that interfered with the fibrinolytic activity of the blood. It has been observed by Barnard,[2] as well as by us, that most of the thrombi that are injected into rabbits lyse and disappear completely. In rabbits killed a short time after the intravenous injection of clot-suspension, the pulmonary arteries are found to contain a large number of thrombi. If rabbits receiving similar quantities of clot are killed a week or longer after intravenous injection with clot, the total number of lesions (thrombi and fibrous intimal thickening) is much smaller than would be expected if all thrombi had organized. Hence, anything that interferes with the lysis of thrombi might increase the yield of fibrous lesions.

The experiments of Cullen and Swank [8] suggest a third possible explanation for the effect of intermittent fatty meals. They observed an increased adhesiveness of red blood cells and aggregation of platelets in the cheek pouch of hamsters following alimentary lipemia. The increased yield of fibrous intimal lesions in the pulmonary arteries of rabbits simultaneously given intravenous thrombi and oral fat may be due to the extension of the thrombi after they became lodged in pulmonary arteries.

It is interesting to note that meals consisting of butter did not produce a significantly greater effect on pulmonary arterial lesions than did meals consisting of oleomargarine. However, it is possible that other types of vegetable oil, such as corn oil, might have an entirely different effect.

Role of Transient or Sustained Pulmonary Hypertension.—Transient or sustained pulmonary hypertension may be a factor in the formation of pulmonary arteriosclerotic lesions associated with thromboembolism. However, pulmonary hypertension resulting from embolization should not affect comparison among the rabbits receiving clots in the

current study, because each of these rabbits received the same amount of clot. Any differences in pulmonary arterial pressures among the rabbits given intravenous blood clot must have been the result, rather than the cause, of the obstructive pulmonary arterial lesions.

The fact that differences in the relative weights of the right ventricle were found in the various groups at the end of the experiment simply tend to confirm the observation that there were differences in the incidence and severity of obstructive pulmonary arterial lesions at the end of the experiment.

SUMMARY

In recent years, a number of investigators have produced fibrous intimal thickening of small pulmonary arteries in rabbits by repeated intravenous injections of blood clot. Absence of fat in the lesions has been reported in almost all instances, although some of the lesions are otherwise characteristic of pulmonary arteriosclerosis.

It is possible that the absence of fat in the sclerotic arteries is related to the paucity of fat in the rabbit's diet. Hence, a series of experiments was planned to determine the effect of fatty meals on thromboembolic-induced pulmonary arterial lesions in rabbits. Rabbits receiving weekly injections of blood clot intravenously were divided into groups and given, in addition, by means of transoral gastric intubations, melted butter ("butter-clot" group) or melted oleomargarine ("oleomargarine-clot" group) or warm water ("water-clot" group).

Microscopic sections of lung from 42 rabbits (15 from the water-clot group, 15 from the butter-clot group, and 12 from the oleomargarine-clot group) were examined. Fibrous intimal thickening in the pulmonary arteries was present in all groups, but the frequency and severity of lesions was significantly greater in animals receiving supplementary oral lipids.

Small but definite quantities of fat were present in almost one-half of the animals in all groups. The unexpected presence of fat in the pulmonary arterial lesions of the water-clot group is of special interest. Similar pulmonary arterial lesions have not been observed in control rabbits receiving only intravenous saline and intragastric butter or oleomargarine.

The explanation for the effect of intermittent fatty meals on the frequency and severity of thromboembolic-induced pulmonary lesions is not clear. One possibility is that, at intervals, more fat had been present in the pulmonary arterial lesions of the rabbits receiving supplementary fats than was present at the time of death and that even the temporary presence of the fat increased the fibroblastic reaction. Another possibility is that the fatty meals could have resulted in temporary alterations that interfered with fibrinolysis and that this permitted some thrombi to remain and organize that would otherwise have been lysed. A third possibility is that the orally administered fat increased the coagulability of the blood and caused extension of the thromboemboli.

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Ischiopagus Tetrapus

Report of a Case

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Ischiopagus, from the Greek words for "hip" and "fixation," is the variety of conjoined twins in which the connection is in the lower pelvic region with the axis of the bodies extending in a straight line in opposite directions. In some instances two legs extend at right angles from each lateral surface (tetrapus), and in other instances there are two legs on one side and fused lower extremities on the other (tripus). There may be two legs on one side and none on the other (bipus). Wilder,[1] writing in 1904, illustrates a drawing of ischiopagus (tetrapus) duplicates born near Frankfort, Germany, at least as early as 1643. Schlumberger and Gotwals [2] state that the earliest authentic case of ischiopagus tripus was reported in 1554.

According to Wilder,[1] these cases were said to be numerous but seldom lived beyond infancy. Several of the early cases were exhibited in Ceylon, France, and this country. On the contrary, according to others the incidence of conjoined twins of any type is very low. Mortimer and Kirshbaum [3] cite Mall, who estimated that on the basis of the collection of about 100,000 cases (of pregnancy) from different observers, about 7% terminated in aborted pathologic ova and about 0.6% in malformed fetuses at term. Of the latter, only a small portion were conjoined twins. Puech, cited by Scammon,[4] examined 100,000 births and found 517 malformations, of which only 2 were double monsters. Potter [5] states that symmetrical conjoined twins are rare, only one specimen having been delivered at Chicago Lying-In Hospital since 1931, among more than 60,000 deliveries. Considering that there are many other types of conjoined twins beside ischiopagus duplicates, the incidence of the latter must be extremely small. According to Scammon,[4] it is very rare. Mudaliar [7] studied nine cases of conjoined twins, four of which he attended in delivery. During the period in which he saw these 4, there were 25,000 deliveries at the clinic in Madras, India. This would appear to be an unusually high incidence of such anomalies. The series of nine cases included only one of ischiopagus.

REPORT OF CASE

The following case is reported because of the rarity of the condition and the unusual symmetry of the component parts. It also illustrates the futility of any surgical attempt to separate such anomalous twins, if born alive.

Clinical Case History.—The mother, a 23-year-old white woman, had had measles, mumps, whooping cough, and chickenpox in early childhood and scarlet fever at the age of 10. She had had two previous pregnancies, the first, in 1950, resulting in a 5 lb. 14 oz. (2665 gm.) girl after 8 hours of labor, and the second, in 1952, in a 6 lb. 2 oz. (2778 gm.) girl after 2 hours and 38 minutes of labor. Both are living and well, with no physical deformities. There was also no history of congenital anomalies in the family of either parent. The last menstrual period before the present pregnancy was May 11, 1954. The prenatal course was not remarkable. Only one fetal heart beat was ever heard during periodic examinations. This was still audible two days before delivery. The patient went into labor Dec. 24, 1954, and 2 hours and 38 minutes later there was spontaneous delivery of premature stillborn conjoined twin girls, the first twin born being in vertex L. O. A. position, the remaining twin following without difficulty. The mother was discharged from the hospital three days later.

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From the Tompkins County Memorial Hospital and the Tompkins County Laboratory, Ithaca, N. Y.

Dr. Philip Robinson supplied the clinical data for this study, and Dr. Murray George did the roentgenogram.

Anatomical Study.—Permission was obtained for an autopsy with hospital disposal, but, because of an anticipated delay in signing of the authorization, the teras was placed in formaldehyde solution pending examination, which was commenced three days after the stillbirth. Hence, all of the dissections were made on fixed, or partially fixed, structures and organs. One twin was somewhat more macerated than the other, and for purposes of identification this twin is designated hereinafter as Twin A, and appears to the reader's right in all illustrations and diagrams.

The premature baby girls were joined at the buttocks, so that each right and left lower extremity was separated by the broad point of union (Fig. 1). Together the twins weighed 2280 gm. after fixation in formaldehyde and measured 41 cm. from the vertex of the head of one to the vertex of the other. Twin A measured 19.5 cm. from its vertex to the centrally placed, single umbilical cord, while the same measurement for Twin B was 22.5 cm. Arms and legs of each infant were well formed and symmetrical. Vaginal orifices were present between the legs on each side of the bodies. Labia between the right leg of Twin A and left leg of Twin B were poorly formed, whereas on the opposite side of the body there were distinct labia majora and minora.

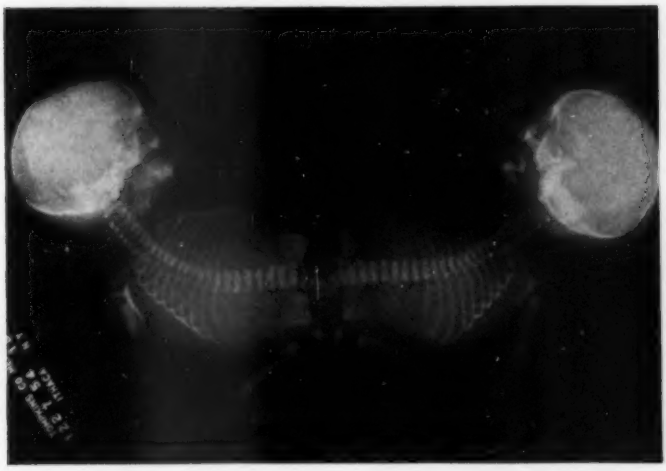

Fig. 1.—Roentgenogram showing relationships of vertebral columns. Note that the coccyx of each twin points to the same side of the body.

Internal organs and structures of the twins will be described together, differences being indicated where found. Organs of both showed much postmortem change. They were not weighed. There was no appreciable amount of fluid in the peritoneal cavities, but a scant amount of reddish fluid was present in each pleural sac. A slight amount of discolored fluid was in each pericardial sac. The thymus of each infant was moderate in size.

The hearts were not weighed, but they were compatible in size with the development of the infants and with each other. The valves were not remarkable. The foramen ovale was patent, as was the ductus arteriosus of each infant. There were no abnormalities of the great vessels at the base of the heart. Lungs, spleen, duodenum, stomach, pancreas, and liver of each twin were not remarkable except for postmortem autolysis. Extrahepatic bile ducts were too small to be adequately dissected out or probed. The left adrenal of Twin A was flat and discoid, but the right had "cocked-hat" appearance. The right adrenal of Twin B was smaller than the right adrenal of Twin A. The organs of the neck were not remarkable except for some degree of merging of the epiglottis with the base of the tongue, more conspicuous in Twin A.

The kidneys of Twin B were not remarkable. Twin A had but one kidney, situated rather low on the right, with its lower pole behind the right side of the uterus (Fig. 2). There were two ureters; the one arising from the anteromedial aspect of the midpart of the organ passed to the right side of the bladder. The other arose medially and distal to this and passed medially and inferiorly to enter the left side of the common urinary bladder. This latter structure formed the pelvic floor for each infant and contained a quantity of brown mushy material. The ureteral orifices could not be probed because of their small size. There was one slit-like orifice of exit, about 0.9 cm. in length, opening into the vaginal tract. The bladder wall was relatively thick.

Each twin had its own uterus, which was closely adherent to the bladder (Figs. 2 and 3), was broad and slightly bicornuate, and had a thin septum dividing the uterine cavity into two parts,

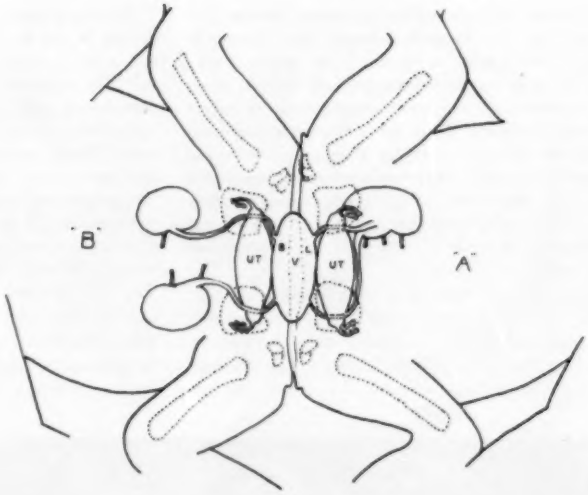

Fig. 2. — Genitourinary systems. *BL*, common bladder; *UT*, uterus; *V*, common vagina. Note single kidney with double ureters of Twin A.

which contained brown mushy material. The right cavity of Twin A was smaller than the left. A vaginal space extended entirely across the conjoined body. The walls of this were fused with the adjacent uteri, the inferior aspects of which were situated close to the vertebral column (Fig. 3). No true pelvic cavity was present. Minute openings less than 0.1 cm. across passed from the sides of the vagina into each uterine cavity, though no opening was demonstrated into the left cavity of Twin B.

The small intestine of each twin was not remarkable. The terminal part of each large intestine was much dilated and contained sticky greenish material. Each communicated with the other low down in the sacral region, near the tip of the sacrum and in its very slight concavity, the orifice of communication narrowing to about 0.4 cm.

The separate vertebral columns fused at the lowest lumbar vertebrae, the common sacrum making a right angle to the longitudinal axis, pointing toward the side of the twins between right leg of Twin B and left leg of Twin A (Fig. 1). Its posterior aspect was open. The posterior longitudinal ligament extended from the lumbar vertebrae of one infant to those of the other.

Vascular systems of each infant (Fig. 4) were much the same, with some variations. The hearts and upper parts of the aortas were not remarkable. In Twin A the abdominal aorta divided into practically equal-sized branches, which passed laterally to the uterus to extend up the posterolateral aspect of the bladder to become the umbilical arteries. The femoral artery on the right originated rather low down, and the internal iliac artery was a small vessel arising distal to this. The right umbilical

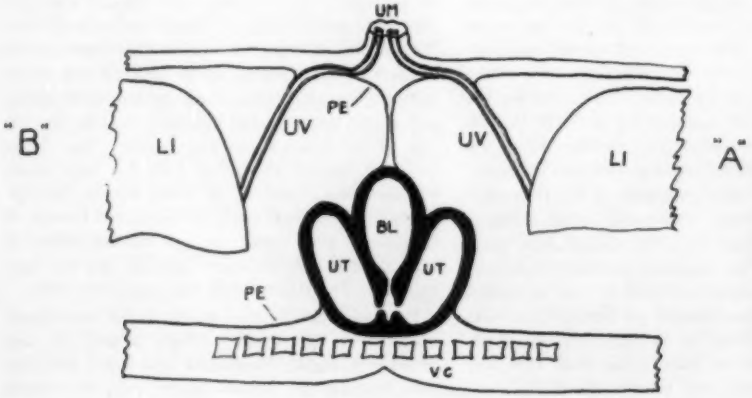

Fig. 3.—Schematic representation of sagittal section of monster. *UM*, umbilicus; *PE*, parietal peritoneum; *UV*, umbilical vein; *LI*, liver; *BL*, bladder; *UT*, uterus; *VC*, vertebral column.

artery became rather small as it entered the umbilical cord. The left external iliac artery arose shortly after the bifurcation of the aorta and had a longer course before it gave origin to the internal iliac artery and became the left femoral artery. The left umbilical artery, as it entered the cord, was larger than the right. There were three right renal arteries, one to each pole and one entering at the hilum.

In Twin B the aorta divided into unequal-sized divisions, the right common iliac artery being at least twice the diameter of the left. It continued to form the right umbilical artery, which passed around the uterus and up the surface of the bladder. The femoral and internal iliac arteries arose from it. The left umbilical artery was small throughout its course, and as it passed along the bladder it became so small that it could not be grossly followed; it did not appear to enter the umbilical cord.

The cord near the monster contained five vessels —two umbilical veins and three umbilical arteries. Further toward the placenta only two arteries were found, the relatively small right artery of Twin A also having disappeared. The lumen of one of the other arteries likewise became rather small. The umbilical veins passed in the usual manner to each liver, bearing the usual relationship to that organ. The vein of Twin A was smaller than that of Twin B and not easily probed. The ductus venosus of each duplicate was patent. The vena cava inferior and its tributaries more or less followed the arteries.

Neither brain showed change of note other than advanced autolysis. The spinal cords were also very soft and discolored. In each infant the sciatic nerves arose bilaterally from the cord and followed their usual courses. The other spinal nerves appeared to have their normal distribution. Sacral nerves were too small to be successfully dissected. The pituitary of each twin was not remarkable.

Microscopic sections from organs of each twin showed pronounced autolysis, and no information was gained by their study.

The placenta was also examined. It measured 15 cm. across and about 3 cm. in thickness. Cotyledons were somewhat torn and varied considerably in size. Only one umbilical cord, 15 cm. in length, was present, inserted near one margin. Microscopically there were small areas of necrosis in the decidual part of the placenta, with a few leucocytes. The umbilical vessels have previously been described.

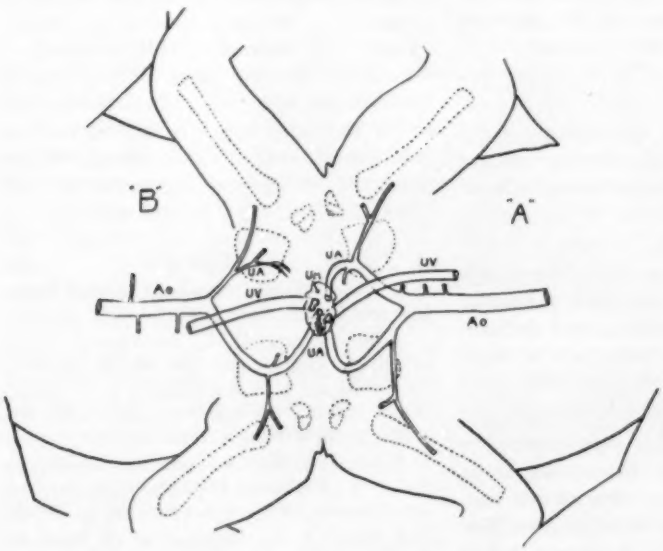

Fig. 4.—Vascular systems. *UA*, umbilical artery; UV, umbilical vein; *UM*, umbilicus; *Ao*, aorta.

COMMENT

No attempt will be made in this article to discuss the embryology of double monsters. This has been well covered in articles by Wilder,[1] Schlumberger and Gotwals,[2] and others.

Detailed descriptions of the internal organs and skeletal and vascular relations of ischiopagus monsters are few. Dunbar[6] cited Dr. John Cooper, who in describing a case stated

that the osseous union was at the "tuber ostis of the ossa ischia and the genital organs and anus of each child are on its right side, between its right leg and the left leg of the other." Potter [5] says of ischiopagus that the bodies are fused in the region of the pelvis as far as the level of the common umbilicus, and above this each is normally developed. The lower ends of the spines are abnormal, and the sacrum and pelvis are often single and directed toward one side. Schlumberger and Gotwals [2] also observed a single fused sacrum at right angles to the rest of the vertebral column, which in the tripus type of anomaly pointed toward the sides of the conjoined bodies from which the two legs arose.

Mudaliar [7] pointed out, in discussing surgical separation of conjoined twins, the deceptiveness of external appearances, which may suggest a superficial union in cartilage or soft tissue, whereas much more intimate union may exist in internal organs such as the heart. The complexity of union in ischiopagi is well demonstrated in Schlumberger and Gotwal's [2] Case I, in which the aortas and venae cavae were united across the midline of junction of the twins and a single fused kidney served both twins. The uteri lay at either side of the conjoined bodies, and it was assumed that half of each belonged to each twin. In their Case II, the venae cavae were continuous and the aorta of one twin was continuous with the right common iliac artery of the other. It is of interest that in their cases, as well as in the one here reported, there was but one bladder, situated in their Case II transversely at the site of junction. Various other anomalies were present in both twins. Mudalier's [7] case had a common abdominal cavity, with the peritoneal lining common and continuous. There were two sets of uteri, tubes, and ovaries, but the plane of the uteri was in the longitudinal axis of the fetuses rather than the transverse, and the single bladder was situated on the side of the two lower extremities.

The case of ischiopagus tetrapus described here is unusually symmetrical as compared with other ischiopagi in the literature, but the impossibility of successful separation of such twins, if living, is obvious.

SUMMARY

A case of ischiopagus tetrapus is reported in which there was an unusual degree of symmetry of the component twins both externally and internally. This symmetry is compared with the more or less chaotic arrangements of organs and vasculature seen in the few other carefully studied cases of ischiopagus found in the literature. The impossibility of surgical separation of such twins, if born alive, is pointed out.

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Studies on Atopic Dermatitis

III. The Effect of Corticotropin, Cortisone, and Hydrocortisone on Storage of Melanin in Basal Cells and on Dopa Oxidase Reaction

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In a previous report [1] it was shown that in active lesions of atopic dermatitis the basal cells of the epidermis fail to store melanin, despite the presence of abundant pigment granules in the intercellular spaces. Furthermore, the melanocytes in the areas of dermatitis reacted more strongly with dopa than those in the uninvolved skin. As acuteness of the dermatitis subsided, the basal cells regained their capacity to store melanin and the melanocytes reacted less intensely with dopa substrate. In view of the known therapeutic effect of corticotropin and the corticoid hormones on atopic dermatitis, it was decided to study their influence on melanin storage in basal cells and on oxidase activity of melanocytes.

METHODS

Biopsy specimens from 12 patients used for the first report of this series [2] were available for this study. These had been obtained from seven women, ranging in age from 26 to 40, and five men, from 21 to 26 years of age; seven were from white and five from Negro patients. The specimens had been obtained as follows: Two morphologically similar sites in an area of dermatitis within a radius of 2 cm. and two corresponding normal areas on the back were selected for biopsy before and after treatment. The latter areas had not been exposed to sunlight for at least six months. Immediately after removal, the skin specimens had been frozen at —150 and —160 C in isopentane cooled by liquid nitrogen and had then been dehydrated *in vacuo* at about —30 C according to the method of Altmann-Gersh.[3] After dehydration, the tissues had been prepared for histologic study by infiltration with paraffin.

For our present study, 10 to 12 sections from each tissue, cut at 4μ and 8μ, were mounted on one slide to permit evaluation of uniformity of technique. After staining with the dopa substrate, preparations of similar thickness were selected for comparison of the intensity of the dopa reaction. The pre- and post-treatment preparations of both affected and normal sites were deparaffinized with xylene and stained simultaneously in the same dish for dopa oxidase activity. The staining technique used was one previously described for semiquantitative evaluation of dopa oxidase activity.[4] It consists of immersing the deparaffinized sections from frozen-dried tissues in a 1:1000 solution of buffered dopa (pH 7.4) at room temperature for 3 hours 45 minutes. Since only melanocytes are stained with the substrate, the same sections may also be studied for preformed melanin distribution.

METHOD OF TREATMENT

The 12 patients included in this study were treated as follows: Of these, 8 received corticotropin, and 10, cortisone or hydrocortisone; 6 of the latter group were included among those who received corticotropin. The interval between different courses of hormone therapy for those who received more than one type of hormone was two to three months, depending on the recurrence of dermatitis.

The interval between the pre- and post-treatment biopsies varied from 10 to 14 days. During this period, the average total dose of each of the three hormones was as fol-

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From the Department of Medicine and the Allergy Unit, University of Illinois College of Medicine.

lows: corticotropin 920 units, cortisone 1210 mg., hydrocortisone 1000 mg. The daily dose given during the first two to four days was high (120 to 160 units of corticotropin, 200 mg. of cortisone, 160 mg. of hydrocortisone per day). After two to four days, depending on response to therapy, it was reduced to about 50% of the initial amount and maintained at this level until the dermatitis showed no further improvement for four successive days. Following the second biopsy, the daily dose was gradually reduced during another two-week period, when the hormone was discontinued. All patients received 2 gm. daily of enteric-coated KCl tablets in divided doses.

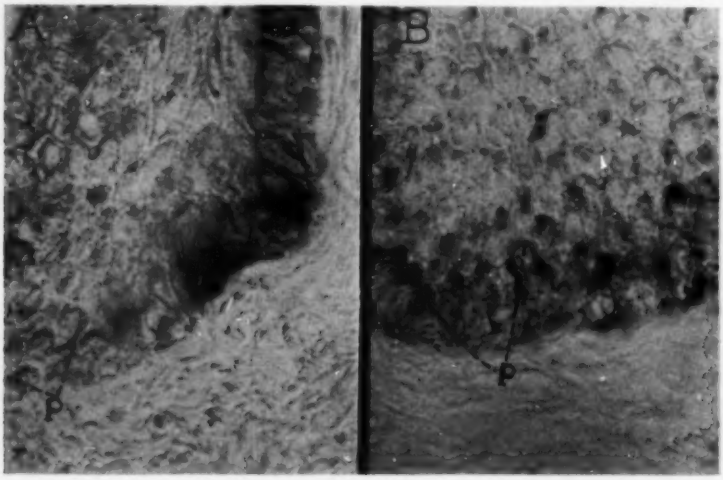


Fig. 1.—Sections from adjacent areas of atopic dermatitis in a Negro patient before (*A*) and after (*B*) treatment with corticotropin. The sections were stained with dopa and photographed at the same magnification; × 360.

Note that in the section before treatment (*A*) the pigment granules (*p*) are outside the basal cells in the widened intercellular spaces. After treatment with corticotropin (*B*) the pigment granules (*p*) are in abundance within the basal cells, appearing as "supranuclear caps" in most cells.

RESULTS

Melanin Distribution.—It was noted, in the second of these studies,[1] that in the presence of intra- and inter-epidermal edema the basal cells contained no melanin despite its abundance in the intercellular spaces. Following treatment with corticotropin, cortisone, and hydrocortisone, inter- and intracellular edema was reduced and melanin was found within the cells of the stratum germinativum. This change was most strikingly seen in Negro patients (Fig. 1*A* and *B*). After therapy an abundance of melanin granules were present within the epidermal cells, often arranged as "supranuclear caps." The dermis, however, contained the same number of melanin-filled chromatophores before and after treatment.

In the normal skin melanin distribution was not altered by treatment with corticotropin or adrenocortical hormones.

Effect of Corticotropin on the Dopa Reaction in Areas of Dermatitis: Only one of the eight patients who received corticotropin failed to show stainable melanocytes with the dopa technique described. He was a red-haired person with very lightly pigmented skin. One patient, a woman with very fair skin, had a 1+ melanocyte reaction which was not altered by corticotropin treatment.

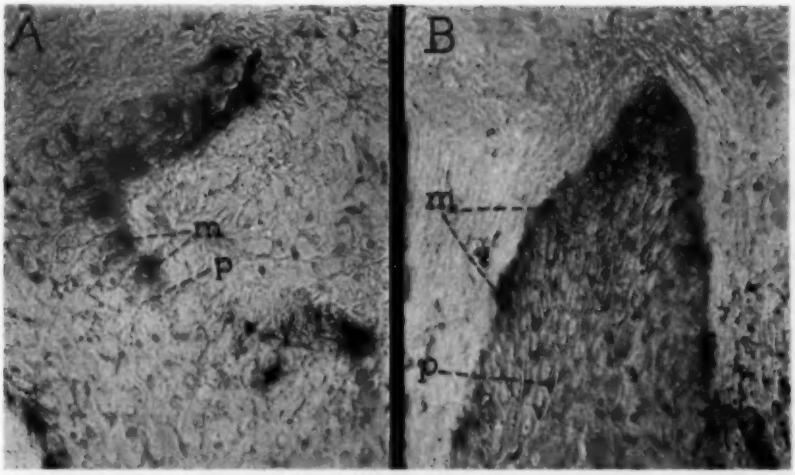


Fig. 2.—Sections from adjacent areas of atopic dermatitis in a Negro patient before (*A*) and after (*B*) treatment with corticotropin. The sections were stained with a semiquantitative dopa method and photographed at the same magnification.

Note the greater staining intensity of melanocytes (*m*) in the section before treatment (*A*) compared with those in the section after corticotropin therapy (*B*).

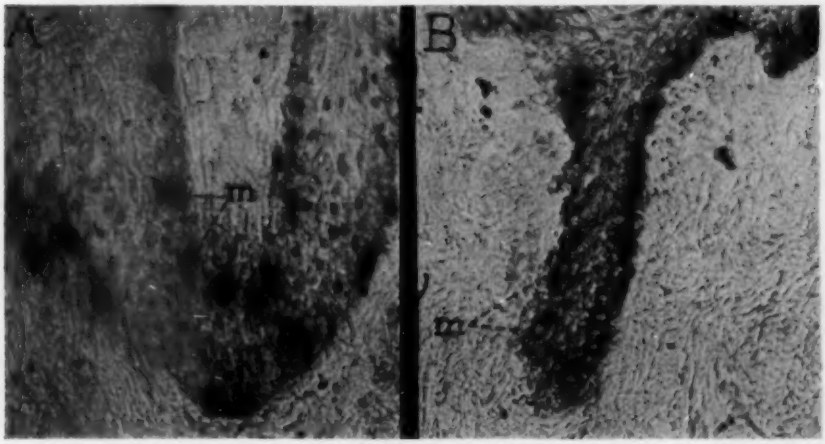
The melanin pigment (*p*) before treatment (*A*) is in the intercellular spaces and not within the basal cells; after treatment (*B*) it is abundant within the basal cells.

The remaining six patients, whose dopa reaction before therapy ranged from 2+ to 3+, showed the following changes: The melanocytes in three, which had been graded 3+ before therapy, were reduced to 1+ after corticotropin treatment (Fig. 2*A* and *B*); and the melanocytes in the other three, which had been graded 2+ before therapy, were altered to 0 in two cases and to 1+ in one case.

Effect of Cortisone and Hydrocortisone on the Dopa Reaction in Areas of Dermatitis:

Fig. 3.—Sections from adjacent areas of atopic dermatitis in a Negro patient before (*A*) and after (*B*) treatment with hydrocortisone. The sections were stained with a semiquantitative dopa method and photographed at the same magnification; × 360.

Note the marked staining intensity of the melanocytes (*m*) in the section before treatment (*A*) and the reduction in the dopa reaction (*m*) after hydrocortisone therapy (*B*).



Either cortisone or hydrocortisone was used in 10 patients. Eight of these had a decrease in dopa reaction following such treatment. One of the remaining two showed an increase in the dopa stain from 1+ to 2+, and the other had no change in the 1+ reaction.

The eight whose dopa reaction was decreased after cortisone or hydrocortisone therapy were grouped as follows: a reduction from 1+ to 0 in one, a reduction from 2+ to 1+ in two, a reduction from 2+ to 0 in one, a reduction from 3+ to 1+ in three.

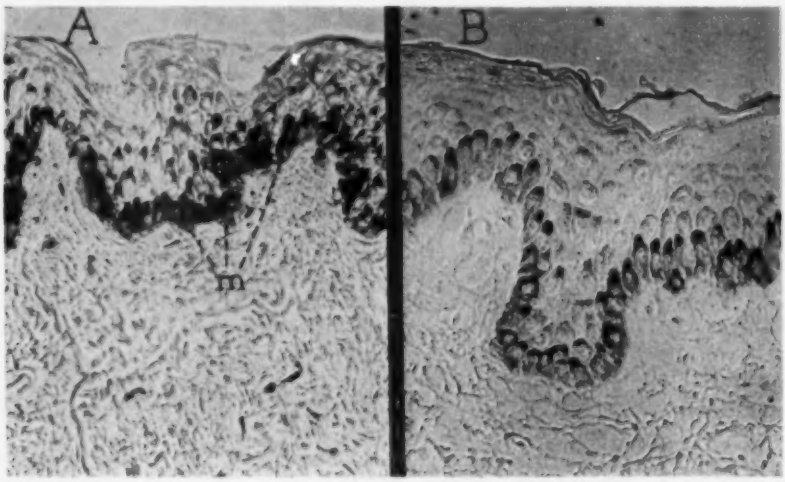


Fig. 4.—Sections of normal skin from corresponding infrascapular areas of a Negro patient before (*A*) and after (*B*) treatment with hydrocortisone. The sections were stained with a semiquantitative dopa method and photographed at the same magnification; × 360.

Note that the melanocytes (*m*) stain with moderate intensity (2+) in the section before therapy (*A*). They can be best seen where they dip into the cutis below the epidermal-dermal junction. In the section after treatment (*B*) no melanocytes with a positive dopa reaction could be found.

One of these last three had received courses of treatment with hydrocortisone and cortisone. The reduction in dopa reaction was from 3+ to 1+ after both materials. In one patient with a 4+ dopa reaction the reduction was to 1+ following hydrocortisone therapy (Fig. 3*A* and *B*).

Effect of Corticotropin on the Dopa Reaction in Normal Skin: One of the eight patients who was treated with corticotropin had generalized atopic dermatitis, so that no normal area was available for examination, and another, the patient with red hair, had a negative dopa reaction in the normal skin. The remaining six were grouped as follows: Five showed definite reduction in the reaction from 1+ before treatment to 0 after therapy; the sixth patient showed no change in the 1+ reaction present before and after treatment.

Effect of Cortisone and Hydrocortisone on the Dopa Reaction in Normal Skin: Nine pairs of biopsy specimens from normal skin sites were available for comparison in this group of patients. One of these remained unaltered after therapy, and eight showed a reduction in the dopa oxidase reaction after treatment. The changes were as follows: Of four with 2+ reaction before treatment, three were reduced to 1+, and one to 0 (Fig. 4*A* and *B*); of four with 1+ reaction before treatment, all were altered to 0 following treatment.

COMMENT

In atopic dermatitis, as in most inflammatory skin conditions, hyperpigmentation is common. Despite increase in pigment formation, melanin granules are not found in the epidermal cells during the acute stage of atopic dermatitis. Masson stated that the melanocytes are "cytocrines" whose dendritic processes are in contact with the Malpighian cells so that the pigment can be inoculated into these cells. The epidermal edema with widening of the intercellular spaces characteristic of the acute phase of atopic dermatitis may break such contact and prevent the flow of pigment from dendrites into epidermal cells. The marked intracellular edema may also prevent the normal flow of melanin and its storage in the cells. With the reduction in the epidermal edema following treatment with corticotropin or the corticoid hormones there is restoration in the normal flow of pigment and its storage within the cell. The continued presence after therapy of many melanin-filled chromatophores within the dermis suggests that despite clinical improvement pigment is still formed in excessive amount and is, in part, excreted into the cutis.

A stronger dopa reaction in areas of atopic dermatitis than in normal skin was to be expected, since it has long been recognized that melanogenesis is increased after skin inflammation.

The small number of patients available for this study permits only tentative conclusions about the effect of corticotropin and adrenocortical hormones on the dopa reaction in melanocytes. There seemed to be a definite trend toward a reduction of the dopa reaction after treatment with these hormones. That this effect occurred in the normal as well as in the involved areas indicates that the reduction in melanocyte activity is due to general lowering in melanogenesis rather than to improvement in the dermatitis alone.

While corticotropin is recognized for its melanotropic as well as for its adrenocorticotropic effects,[6] it would appear, from the small group included in this study, that the latter influence is the stronger at the dose used (about 900 units over a two-week period). Presumably, the corticoid hormones resulting from a relatively high dose of corticotropin over a brief period are more effective in suppressing melanocyte activity than is the intermedin effect of the administered corticotropin in stimulating melanogenesis.

Reduction in the dopa reaction following the use of corticosteroids is probably attributable to inhibition of pituitary intermedin by adrenal hormones. It is not surprising that, following therapy with corticotropin or corticosteroids, an occasional patient showed no change or even an increase in melanocyte activity. It is likely that melanocytes of different persons vary greatly in response to stimulation. There is considerable variation in the tendency to melanosis in patients receiving even large doses of corticotropin over long periods. And, while it is uncommon for cortisone or hydrocortisone to produce gross increase in pigmentation,[7] an occasional patient may show either diffuse or localized melanosis after prolonged steroid therapy without previous treatment with corticotropin.*

SUMMARY AND CONCLUSION

Skin biopsies from 12 cases of atopic dermatitis were obtained from involved and uninvolved skin before and after courses of treatment with corticotropin, cortisone, and hydrocortisone. Studies of melanin distribution and of the dopa reaction showed the following:

(1) Restoration of the capacity of the basal cells in the areas of dermatitis to store melanin.

(2) Reduction in most cases in the intensity of the dopa reaction in the areas of dermatitis and in the normal skin following treatment with corticotropin, cortisone, and hydrocortisone.

* Rosenberg, E. F.: Personal communication to the author.

Dr. Francis E. Senear, Professor of Dermatology, Emeritus, University of Illinois College of Medicine, selected patients from his service for this study; Dr. Samuel M. Bluefarb, attending physician, and Dr. Leonard Hoit, resident, Dermatology Department, Cook County Hospital, selected patients from their service for this study.

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Note on Origin of Some Fragments of Bone in Lungs of Laboratory Animals

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P. YEVICH, B.S.
and
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INTRODUCTION

In articles on diseases of laboratory animals,* it has been shown that, apart from common enzootic and epizootic disorders, a motley of incidental lesions of diverse natures, causes, and distribution will be found at postmortem examination, if a search is made. Some may be clinically silent, but are still of great importance (e. g., some stages of chronic murine pneumonia [3] and lung mites in rhesus monkeys [2]). We have encountered others of a bizarre nature which cannot be ignored, however trivial they may appear at first glance, e. g., dystrophic calcification of the adrenal.[4]

At the outset, it should be stressed that we are not dealing with bony deposits in the lungs which are in parallel with the miliary ossification occurring in human lungs in some cases of mitral stenosis [see Elkeles and Glynn (1946)[8]; Lendrum and co-workers (1950)[9]; Sahn and Levine (1950),[10] and Hadfield (1953)[11]]. The human lesions were disseminated in the lower lung field and formed casts up to 4 mm. in diameter. Elkeles and Glynn suggested that they were a late result of the changes following rheumatic fever, that the bone formation took place in the pulmonary exudate, and that there was no surrounding fibrosis.

BONY FRAGMENTS IN THE LUNGS OF ANIMALS

A minute fragment of bone was found first in the lung of a rat, and because it might have borne some analogy to the human lesions, a search was made of the many sections of rodent lungs in our collection. The results were of interest; similar particles of bone were found in the lungs of 7 out 589 rats, 6 out of 315 hamsters, 1 out of 126 rabbits, and 1 out of 245 guinea pigs, but we have not seen them in mice.† In all the thousands of lungs of dogs examined by one of us (J. R. M. I.) none have been seen, but bony morsels have been found in one dog by a worker in our department. Yet, osseous lesions are known to occur in old dogs (see Nieberle and Cohrs, 1952[12]). (The total number of animals examined is irrelevant, because the search was stopped arbitrarily.) Since there were no pathologic differences, a short general description suffices.

In most cases there was only one, two, or three fragments found in the routine slice, made in a horizontal plane, of the rodent lungs. They measured approximately 70μ to 150μ, and were easily identifiable under a low power of the microscope. The particles were lodged in the alveolar spaces, squeezing

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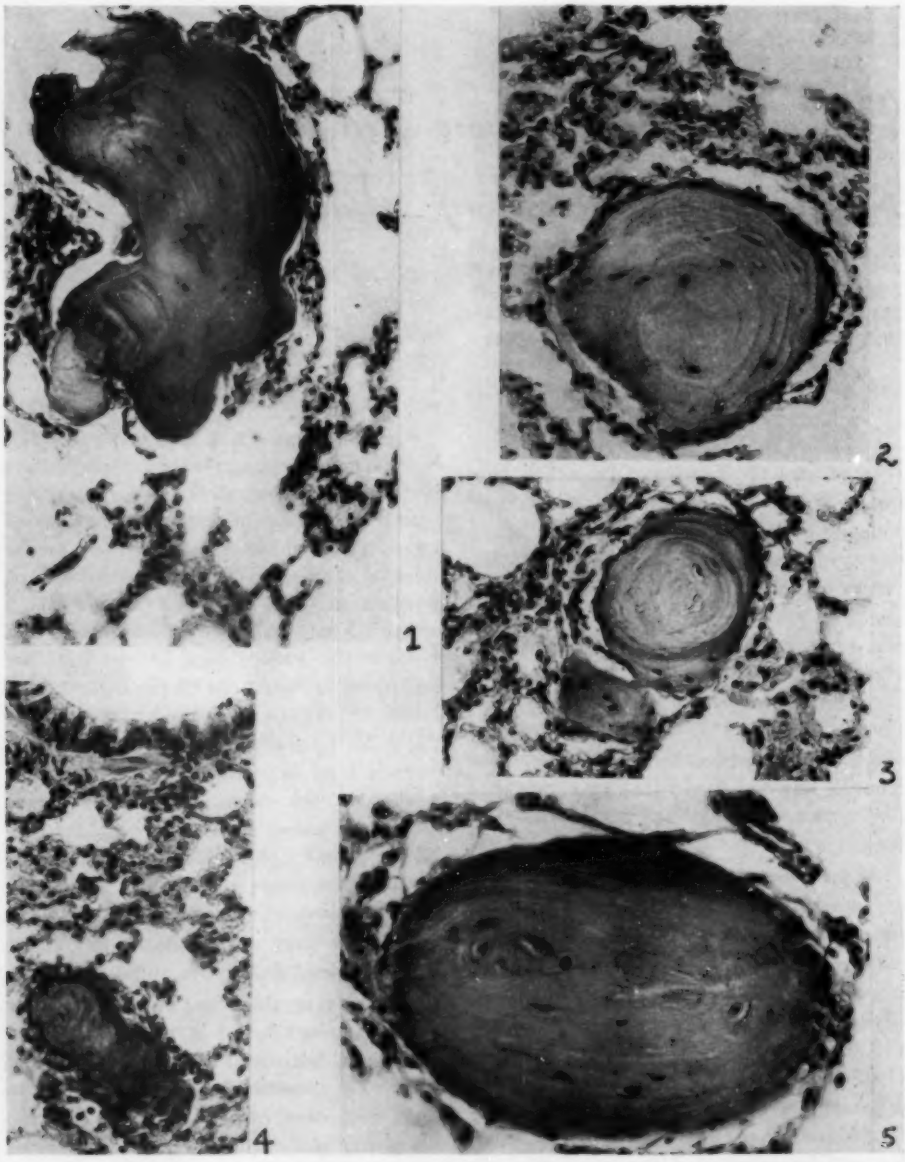
Pathology Branch, Medical Laboratories, Army Chemical Center, Md.

* References 1 through 7.

† Dr. Thelma Dunn, National Cancer Institute, National Institutes of Health, Bethesda, Md., has informed us that she saw spicules of bone in mice infrequently some years ago.



Figs. 1-5.—Bone fragments in lungs, natural cases. Hematoxylin-eosin; × 381. Figure 1, rat (585/55); Figure 2, rabbit (311/55); Figure 3, guinea pig (435/55); Figure 4, hamster (490/54), and Figure 5, dog (861/55). All show similar appearance, with lodgement in alveolar spaces, no reaction or fibrosis.

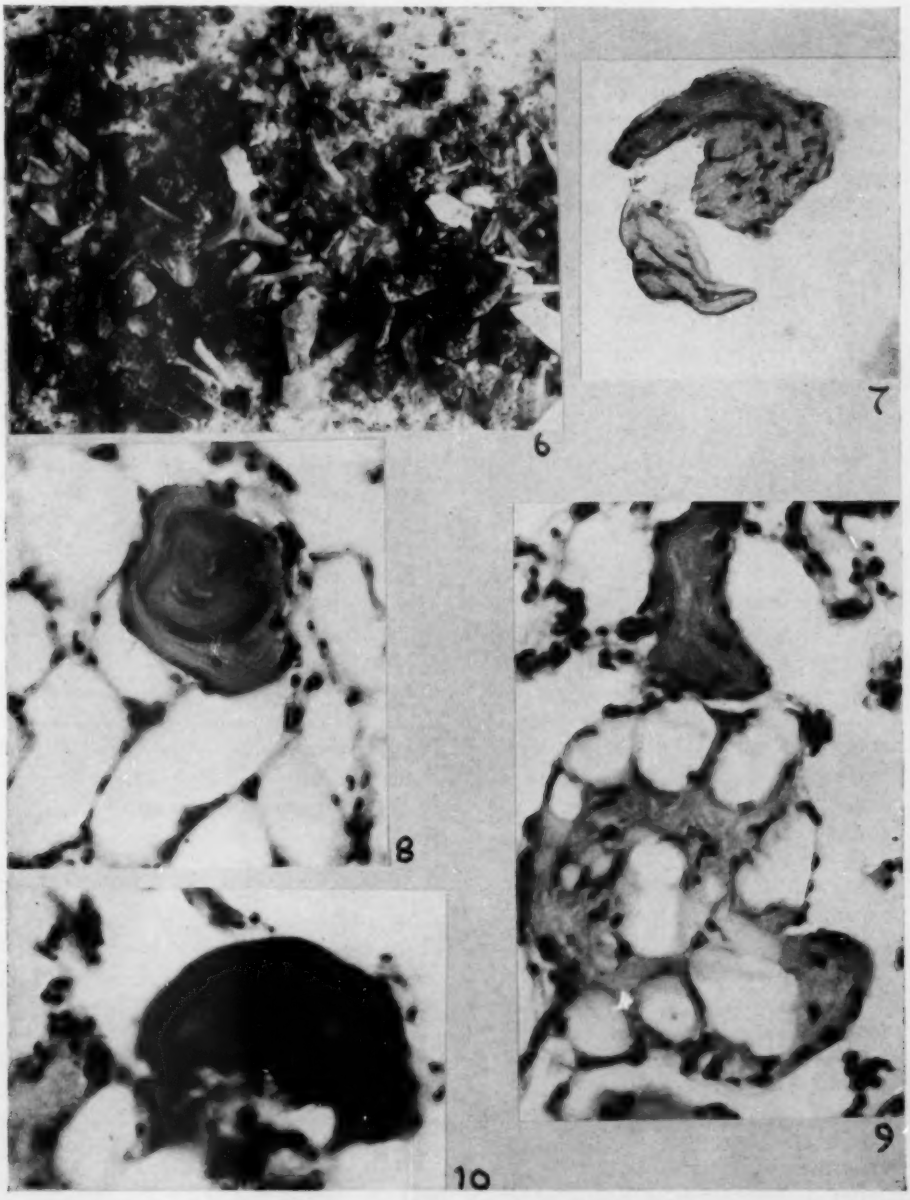


Fig. 6.—Fish meal used in making pellets. Appearance of random sample under dissection microscope; note numerous anguliform and spicular fragments of white bone. × 5.

Fig. 7.—Fragment of bone from section of food pellet. Hematoxylin-eosin; × 381.

Figs. 8-10.—Experimental bone fragments in lungs. Rats exposed to dust cloud stirred up from fish meal in glass chamber. Figure 8 (1116/55 A), rat killed immediately; Figures 9 and 10 (1116/55 B), lungs of rats killed three weeks after exposure. Hematoxylin-eosin; × 381. In Figure 9 there is more than bone, but what is it—scales? cartilage? or what?

aside the alveolar walls, with the latter being molded on the bone. There was no surrounding reaction of any variety—inflammatory cells or fibrosis—and in most animals the particles were present in otherwise normal lungs. In fact, some of the fragments were present in the lungs of rats in a colony bred free from pulmonary, or any other, disease. The resemblance to bone is indisputable, but it should be noted that there are no periosteum, no Haversian systems, and no blood vessels; however, there are osteocytes with a spindle-shaped nucleus, surrounded by a halo. Sometimes the particles were basophilic, and sometimes they were stained pink with eosin as in decalcified bone, and they were birefringent under the polariscope. There was no predilection for the particles to be in the subpleural, or any other, zone, although sometimes they were found in the former location. In some the resemblance to bone was almost lost, and with calcareous deposition present in a disintegrating center, and a few appeared more like highly cellular cartilage. Most, however, were similar to those illustrated (Figs. 1-5). In substance, the fragments are inert foreign bodies in the air spaces and not, strictly speaking, a lesion within tissue at all.

COMMENT

Intrapulmonary osseous fragments are not mentioned as occurring in the lungs of rodents in Jaffé (1932).[13] Nieberle and Cohrs (1952)[12] refer to disseminated plaques of true lamellar bone in the lungs of old dogs, a condition which clearly is not related to the condition described, although it might be analogous to the human lesions referred to above. A single bony gobbet was seen in the lungs of one dog (Fig. 5), however, similar in size and appearance to those in the rodent lungs but which might not have the same origin. The possibility of aspiration of mammalian bone by dogs might also be considered.

As the lungs were mostly free from disease of any variety or contained lesions with which we are highly familiar, and in which neither calcification nor ossification occurrs, it was a challenging puzzle to solve the origin of the bone. Any idea that it arose from exudate, as suggested for the human cases, was dismissed as nebulous, for the cellular components must have arisen originally from osteogenic tissue. Being deeply embedded in tissue and in the same focal plane as the rest of the section, the fragments were clearly not artifact, by being dropped on the section or picked up during routine preparation of blocks. It was therefore contemplated that the fragments were the result of a highly fortuitous aspiration accident, and a few simple observations provided proof.

All our laboratory animals are fed a nationally known pelleted diet, and the one given to rats and hamsters contains 5% fish (menhaden) meal (according to the makers). A sample of the meal was obtained, and sections were made of both the pellets and the fish meal, and bony splinters were recognized without difficulty. In both they appeared no different from those seen in the animal lungs—in size, in irregular shape, and in absence of Haversian systems, which is apparently characteristic of the piscine skeleton. The fish meal, after treatment with potassium hydroxide, then washing and drying, contained approximately 13% dry weight of ground bone (or cartilage and scales, i. e., any material not dissolved by the hydroxide). Examination of the fish meal under the dissection microscope showed that it was not really a fine powder, and that spicules, fragments, or gobbets of bone and cartilage were readily recognizable.[6] However, if stirred up by an air blower in a glass chamber, a dust cloud was easily produced.

THE EXPERIMENT WITH BONE DUST

Four rats, therefore, were exposed in a small chamber, the animals resting on a perforated tin, and the floor of the chamber beneath being covered about 1 in. deep with the fish meal. A strong current of air was played on the meal, and the rats were exposed

to the dust cloud produced for one hour. One rat was killed immediately and the others after three weeks, and many sections of the lungs were examined. Isolated fragments of bone were found in the lungs of all four rats and were identical with those seen naturally, and there was no concomitant pulmonary disease. In the rat killed at one hour, there was also much microscopic vegetable matter deposited in the air spaces, which is not surprising. Just why the bone fragments should remain, and other inert particles disappear is not clear to us, but it does obviously happen. We thus considered the problem solved, and that these microscopic bodies were aspirated fragments of bone derived from powdering of the pellets in the cage, or more likely in the atmosphere of the animal rooms (where sometimes two to three different species were kept). It manifestly must be a singularly fortuitous event. However, bearing in mind the small per cent (5) of fish meal in the pellets, the amount of bone (about 13%) in the meal itself, then the finding of isolated single, or a few, particle(s) of bone in the lungs of such a very small number of laboratory animals should not cause raising of the eyebrows. For the skeptics, we would then ask what other explanation can there be. Pathology is the study of cause, and, with the intervention of time, an effect, and we have demonstrated this sequence of events, if not with complete clarity, then with weighty presumptive evidence.

The condition may be entirely without significance regarding the health of laboratory animals, but all dust diseases are of importance to industrial medicine. We might then well raise an issue whether workers in fish and bone meal factories have ever known to suffer from an "osseous pneumoconiosis." The fact that there was no reaction, or fibrosis, need cause no anxiety, for we know how difficult it was in the past to produce "pneumoconiosis" in the experimental animal, even after many months' exposure. Further, the particles were all still lodged in air spaces and not in tissue at all.

SUMMARY

Microscopic fragments of bone have been found as a rare event in the air spaces of the lungs of rats, hamsters, a rabbit, and a guinea pig, but not mice. There is no surrounding reaction to the inert foreign bodies and no concomitant pulmonary disease. The "lesions" are not analogous to those in human lungs of some cases of mitral stenosis or to those occasionally seen in the lungs of old dogs. Evidence is presented to show that the origin is from aspirated fish bone particles derived from the powdered dust of pelleted diets, most of which contain some fish meal.

ADDENDUM

Curiously, since writing the article, we have found bony fragments in 37 out of 161 rats which had been fed the same diet and were part of a special study involving examination of the lungs.

Mr. John Cuculis, of the Pathology Branch, did the photographic work.

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Experimental Mucormycosis (Rhizopus Infection) in Mice

The Failure of Chronic Alloxan Diabetes to Modify Host Susceptibility

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Human cases of mucormycosis have been observed with increasing frequency in recent years.* Of the predisposing diseases, diabetes mellitus has occurred most frequently. Fungi of the genus Rhizopus have now been cultured from two of the reported human cases,† and it may well be that Rhizopus is the most frequent causative agent of mucormycosis. Rhizopus is a genus of the family Mucoraceae of the class Phycomycetes.

Experimental mucormycosis caused by R. oryzae and R. arrhizus has already been induced in normal rabbits and in rabbits rendered diabetic by alloxan.‡ These studies have demonstrated that during the first several days of the alloxan diabetes the experimental infection in rabbits is fulminating and severe, with vascular invasion, like that observed in rapidly fatal human cases of mucormycosis. After the termination of the acutely toxic alloxan diabetes and the establishment of the chronic diabetic state with hyperglycemia, the experimental infection is scarcely more invasive than in nondiabetic rabbits.[6]

Our study describes experimental infection of mice with R. arrhizus. These experiments in mice were carried out before it was known that alloxan diabetes enhanced susceptibility to Rhizopus infection only in the first several days of the diabetes; we had no mice in this stage. Our results indicate that chronic alloxan diabetes does not alter the experimental infection in mice.

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* References 1 through 4.

† References 1 and 2.

‡ References 5 and 6.

MATERIALS AND METHODS

Subcultures of R. arrhizus from the human case described by Harris[2] were grown on Sabouraud's glucose-agar for four to five days at room temperature. The aerial mycelium with its spores was harvested free of the agar and ground in a sterile mortar with sterile isotonic saline. This produced a black liquid, which was then centrifuged and adjusted to a 10% suspension of the packed fungus in the saline. The resuspended fungus was found on microscopic examination to consist of fragments of brown hyphae, sporangia, and spores.

Mice were rendered diabetic by the injection into a tail vein of 100 mg. of alloxan monohydrate per kilogram of body weight (0.1 mg/gm.), according to the method of Waisbren.[7] A 2% solution of alloxan monohydrate in sterile distilled water was used.

Blood-sugar levels were determined by the method of Reinecke,[8] using 0.02 ml. of blood from the tail vein. Normal mice after fasting 18 to 20 hours were found to have blood-sugar levels of from 75 to 150 mg. per 100 cc. The mice injected with alloxan developed blood-sugar levels of 200 to 550 mg. per 100 cc. within 48 to 72 hours after injection. Our criterion of diabetes was a blood-sugar level of more than 250 mg. per 100 cc. Mice selected for our experiment were those with blood sugar values of over 350 mg. per 100 cc. determined six to eight days prior to inoculation of the fungus. To check

on the maintenance of the hyperglycemia, spot tests of blood sugar were performed on the infected animals on the day of killing (Tables 2 and 4).

Groups of normal and diabetic female mice, weighing 15 to 25 gm. each, were inoculated either intracerebrally with 0.025 ml. or intraperitoneally with 1.0 ml. of the 10% suspension of R. arrhizus.

Except for two mice which died spontaneously the animals were killed with illuminating gas after 1 to 38 days. All autopsies were performed immediately. Fixation was in 10% formaldehyde; staining was by hematoxylin and eosin and by the method of Gridley.[9]

RESULTS

Intraperitoneal Inoculation of Normal Mice (Table 1)

The mouse of this group which died spontaneously, four days after inoculation, presented black plaques on the peritoneal surfaces on gross examination. Microscopically, these foci consisted of the brown hyphae and spores of the original inoculum, with mild proliferation of new colorless hyphae at the edges of the plaques. The fungus deposit was infiltrated with polymorphonuclear cells. There was adjacent fat necrosis, but no fibroblastic response. New proliferating hyphae were found in the superficial portions of the liver, spleen, pancreas, and kidney. In the liver and kidney the numerous hyphae were accompanied by polymorphonuclear cells.

Table 1.—*Rhizopus Arrhizus in Mice*
Intraperitoneal Inoculation of 1 Ml. of a 10% Ground Suspension of Mycelium in 0.85% Saline

Mouse No.	Interval from Inoculation to Death, Days	Extraperitoneal Lesions
1	4*	Liver, spleen, kidney, pancreas
2	6	Liver, kidney, pancreas
3	7	Liver, kidney, pancreas
4	13	Liver, pancreas
5	27	0
6	37	Liver, pancreas

* Died. All other mice were killed.

The mice killed from 6 to 37 days after inoculation presented uniform gross lesions in the peritoneal cavity. There were one or more brown or greenish-brown nodules, 0.5 to 1.0 cm. in diameter, to which the viscera were adherent. The nodules were commonly

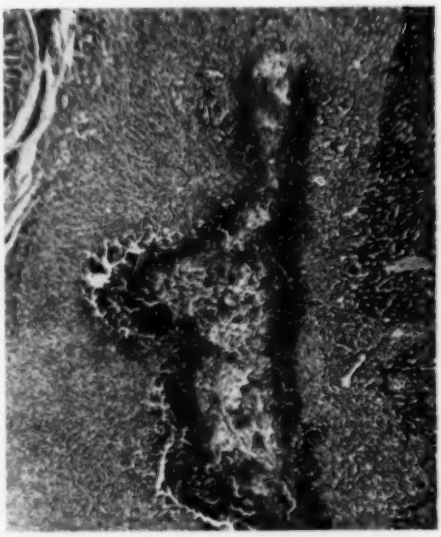


Fig. 1.—Peritoneal nodule from a mouse killed 7 days after intraperitoneal inoculation of R. arrhizus. The nodule consists of the central inoculum of spores and hyphae, peripheral fibrosis, and intervening inflammatory cells. It lies between liver and intestine. Hematoxylin and eosin; × 35.

Fig. 2.—Higher magnification of section of the nodule in Figure 1. The center of the nodule is to the left, where hyphae and spores are seen. Vertically in the center there is an area of necrotic polymorphonuclear leucocytes, and to the right, a zone of fibrosis. Hematoxylin and eosin; × 350.

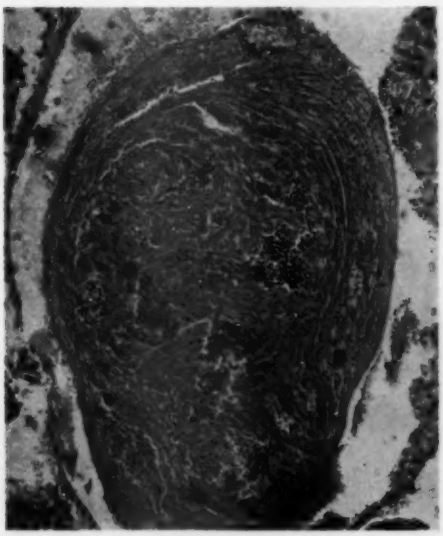
between the spleen, pancreas, stomach, and liver, and occasionally in the pelvis.

Microscopically, these peritoneal nodules were found to contain a central mass of the original inoculum, consisting of brown hyphae, spores, and sporangia (Fig. 1). At the periphery of the inoculum there were colorless proliferating hyphae (Fig. 2), polymorphonuclear reaction, and fibroblastic encapsulation, with giant cells, which sometimes contained brown spores. In the mice killed after 27 and 37 days there was little or no hyphal proliferation, many surrounding macrophages and giant cells, and the fibrous capsule had become collagenous.

Extraperitoneal lesions were present in all the mice except the one killed 27 days after injection. Renal abscesses containing hyphae were present in two mice, while hepatic necroses with hyphae and thrombi in hepatic veins were noted in one mouse (Figs. 3 and 4). The pancreas was usually invaded by the fungus. No fungus was demonstrated in lungs or brain.

Peritoneal nodules from mice killed 13 and 27 days after inoculation yielded R. arrhizus on culture.

Fig. 3.—Thrombus in vein of liver from a mouse inoculated intraperitoneally with R. arrhizus, six days prior to killing. Hematoxylin and eosin; × 60.



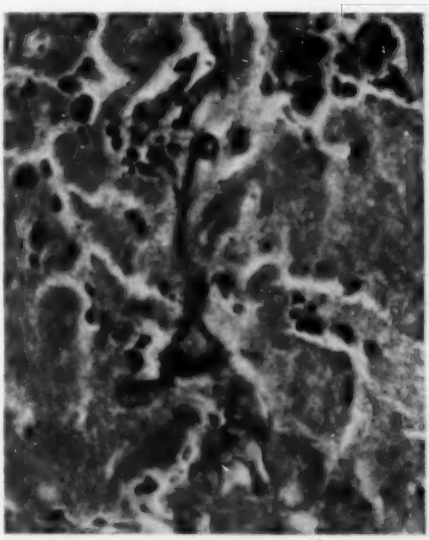


Fig. 4.—Higher magnification of thrombus shown in Figure 3. The hyphae can be seen as broad filaments lying vertically in the center of the picture. Hematoxylin and eosin; × 900.

In brief, inoculation of a 10% suspension of ground R. arrhizus into the peritoneal cavities of normal mice resulted in mild proliferation of the fungus, with frequent extension to kidney, liver, spleen, or pancreas. Recovery of the viable organism from lesions was possible 27 days after inoculation. There was inflammatory reaction in the peritoneal cavity, characterized by progression from a polymorphonuclear response to macrophagic and giant-cell, fibroblastic, and finally collagenous response; local invasion of organs by proliferating hyphae with abscess formation and tissue destruction, in some cases; rarely, thrombi and infarcts. There was no generalized systemic dissemination.

Intraperitoneal Inoculation of Mice with Chronic Alloxan Diabetes (Table 2)

These mice had chronic alloxan diabetes of six to eight days' duration. Grossly, at autopsy, there were small collections of the inoculated fungus in the peritoneal cavity in the mice killed two days after infection, while nodules were found in the peritoneal cavities of mice killed after longer intervals.

TABLE 2.—*Rhizopus Arrhizus in Mice with Chronic Alloxan Diabetes*
Intraperitoneal Inoculation of 1 Ml. of a 10% Suspension of Ground Mycelium in 0.85% Saline

Mouse No.	Blood Sugar, Mg. %: 6-8 Days Prior to Inoculation of Fungus	Blood Sugar, Mg. %: On Day of Killing	Interval, in Days, from Injection of Fungus to Killing of Mouse	Extraperitoneal Lesions
1	425	357	2	Liver, pancreas
2	385	540	2	Liver, spleen, pancreas
3	545	...	6	Liver, spleen, pancreas
4	370	...	6	None
5	385	485	10	Liver
6	345	...	13	Liver, kidney
7	345	...	37	None

Microscopic examination two days after the injection of fungus revealed the inoculum to be infiltrated by polymorphonuclear cells as it lay against peritoneal surfaces. The superficial portion of the pancreas was necrotic and infiltrated with the colorless proliferating hyphae. Invasion of liver consisted of hyphae in sinusoids, and in one mouse, in veins with thrombi.

Mice killed at six days presented peritoneal masses, with necrosis of the surface of the liver in one mouse. Fibrosis had begun. One mouse presented small abscesses containing hyphae in the kidney and liver. The other mouse had no extraperitoneal extension.

A mouse killed at 13 days showed peritoneal nodules with fibrosis. There was necrosis of an entire kidney with numerous hyphae in it and abscesses containing hyphae in the liver.

A mouse killed 38 days after inoculation presented an encapsulated peritoneal nodule with central necrosis and bacteria but no identifiable fungus.

All mice in this group failed to show dissemination of the fungus to thoracic or cranial viscera.

Our impression was that there were no significant differences in the invasiveness or dissemination of the fungus in the diabetic group of mice as compared with the normal group.

INTRACEREBRAL INOCULATION OF NORMAL MICE (TABLE 3)

The mouse that died before the end of a day showed only a mass of the injected fungus in the meninges, without inflammatory response.

At four days there was inflammation of a portion of the choroid plexus with brown spores in a giant cell and with new proliferating hyphae and polymorphonuclear cells.

At six days the brain was partially soft in the gross. Microscopically, an area of encephalomalacia showed colorless hyphae, with polymorphonuclear cells about blood vessels (Fig. 5). The ventricles contained the injected fungus with polymorphonuclear cells. A meningitis occurred in a few regions in the form of fibroblastic plaques incorporating brown hyphae and spores. At nine days the appearances were similar.

At 13 days there was a nodule in the brain consisting of the injected inoculum of brown spores and hyphae (Fig. 6). There were colorless proliferating hyphae at the edge of the inoculum, then a rim of polymorphonuclear cells and macrophages, and further out a fibrous capsule. This lesion was continuous with a focal fibroblastic meningitis. Brown spores were present in macrophages and giant cells. At 20 days the findings were

TABLE 3.—*Rhizopus Arrhizus in Mice*
Intracerebral Inoculation of 0.025 Ml. of 10% Ground Suspension of Mycelium in 0.85% Saline

Mouse No.	Interval Following Inoculation, Days	Findings
1	1*	Fungus in meninges; no inflammation
2	4	Proliferating hyphae in choroid plexus with polymorphonuclear reaction; brown spores in giant cell
3	6	Meningoencephalitis with perivascular hyphae and encephalomalacia; fibroblastic response about brown spores
4	9	Meningoencephalitis with perivascular hyphae and encephalomalacia; fibroblastic response about brown spores
5	13	Fibrous encapsulated mass of inoculum in brain
6	20	Fibrous encapsulated mass of inoculum in brain

* Died. All other mice were killed.

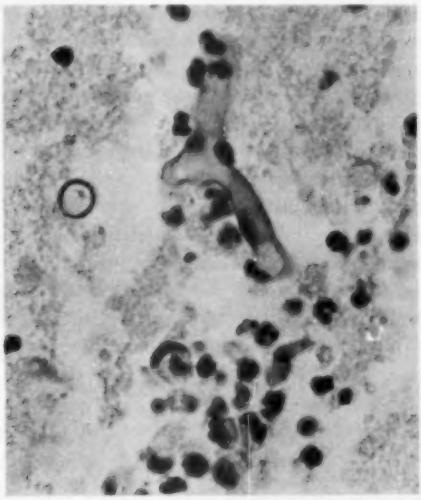
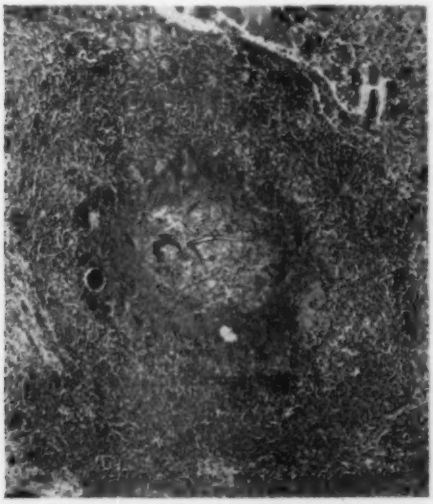


Fig. 5.—Area of encephalomalacia from the brain of a mouse inoculated intracerebrally with R. arrhizus six days prior to killing. A broad branching hypha and a hypha in cross section are seen in the center of the field amidst polymorphonuclear leucocytes and necrotic brain. Hematoxylin and eosin; × 900.

the same, except that no colorless, proliferating hyphae were seen (Fig. 7). Cultures from the brain of the 20-day mouse were positive.

Fig. 6.—Encapsulated abscess in brain from a mouse killed 13 days after intracerebral inoculation of R. arrhizus. Hyphae and a sporangium noted centrally. Hematoxylin and eosin; × 44.



In brief, inoculation of 0.025 ml. of the suspension intracerebrally into normal mice resulted in mild proliferation of the fungus, with viable organisms recovered by culture as late as 20 days after inoculation; an inflammatory response at the site of deposit within the brain characterized by polymorphonuclear reaction, macrophagic and giant-cell response, and fibrous encapsulation; meningitis, ependymitis, and inflammation of the choroid plexus in some cases, and, in one case, a localized encephalitis with encephalomalacia; no systemic dissemination of the fungus.

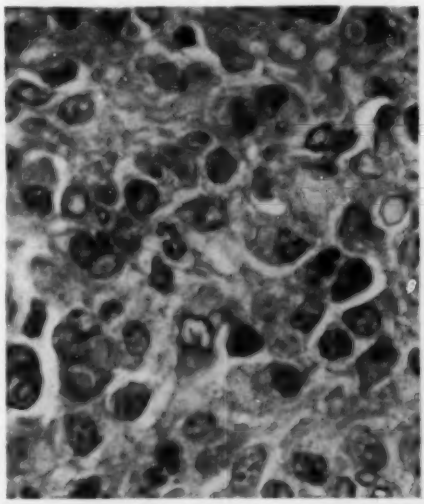


Fig. 7.—A focus of macrophage response in the brain of a mouse inoculated intracerebrally with R. arrhizus 20 days prior to killing. Spores are seen within a macrophage in the right lower corner of the picture. Hematoxylin and eosin; × 900.

Intracerebral Inoculation of Mice with Chronic Alloxan Diabetes (Table 4)

Grossly, the brains of these mice were not remarkable.

Microscopically, the brain of Mouse 1, killed two days after injection, presented two minute infarcts associated with proliferating hyphae in vessels of precapillary size (Fig. 8). This was the only example of vascular involvement.

Mouse 2, killed two days after injection, showed the injected spores and hyphae in the meninges with polymorphonuclear cells

Fig. 8.—An infarct in the brain of a diabetic mouse killed two days after intracerebral inoculation of R. arrhizus. Higher magnification revealed hyphae in the thrombosed vessel just above center in the infarct. Hematoxylin and eosin; × 60.

and fibrin. In one region there were a few proliferating hyphae.

Mouse 3, killed at six days, presented brown spores and hyphae, rarely colorless proliferating hyphae, in the ventricles, with an exudate of polymorphonuclear cells and macrophages. Fibroblasts had begun to proliferate.

Mouse 4, killed at six days, presented foci of meningitis with brown spores, polymorphonuclear cells, and fibroblasts.

The mice killed at 10 days presented ventricles containing macrophages and giant cells with brown spores and a few polymorphonuclear cells and fibroblasts. The same findings were seen in the 13-day mouse. The mouse killed 38 days after injection presented meningeal foci with spores in giant cells.

Our impression was that intracerebral injections of the fungus into mice with chronic alloxan diabetes gave results essentially similar to those observed in the nondiabetic mice. The one instance of intravascular involvement in a diabetic mouse was interpreted as a chance occurrence dependent upon the site of deposition of the fungus rather than upon a difference in host susceptibility.

COMMENT

No Increase in Susceptibility in Chronic Diabetes.—The ineffectiveness of chronic diabetes to increase host susceptibility in the mouse does not specifically conflict with the fact that the infection is fulminating, and often fatal, in the diabetic human. Cases in the human have developed in those diabetics who are in diabetic acidosis and frequently in a terminal state; a case has been arrested by regulation of the diabetes.[2] The mice presented no symptoms of coma when infected, though the levels of blood sugar were characteristic of diabetes. Work by Bauer and co-workers [5] and Elder and Baker [6] indicates that there is much greater spread and destructiveness by Rhizopus in rabbits during the acute toxic stage of alloxan diabetes, i. e., in the first several days after alloxan injections, when there is acidosis. We have not studied the infectivity of Rhizopus during the acute toxic stage of alloxan diabetes in mice.

TABLE 4.—*Rhizopus Arrhizus in Mice with Chronic Alloxan Diabetes*
Intracerebral Inoculation of 0.025 Ml. of 10% Ground Suspension of Mycelium in 0.85% Saline

Mouse No.	Blood Sugar, Mg. %: 6 or 7 Days Before Inoculation	Blood Sugar, Mg. %: On Day of Killing	Interval, in Days, from Injection of Fungus to Killing of Mouse	Findings
1	545	...	2	Infarcts due to hyphae in vessels
2	385	540	2	Acute meningitis with proliferating hyphae
3	440	...	6	Acute ependymitis with proliferating hyphae and fibroblasts
4	465	...	6	Meningitis and fibroblasts
5	385	465	10	Fibroblastic ependymitis
6	500	500	10	Fibroblastic ependymitis
7	544	...	13	Fibroblastic ependymitis
8	415	...	38	Meningeal fibrosis with spores in giant cells

Rhizopus a Relatively Nonvirulent Fungus.—The failure of spread of Rhizopus to the lungs following intraperitoneal injection into mice is in contrast to the ready spread of Blastomyces dermatitidis to the lungs following intraperitoneal injection.[10] This may be a reflection of the relative nonpathogenicity of R. arrhizus under ordinary circumstances, as contrasted with B. dermatitidis. However, our study indicates that R. arrhizus is capable of invading blood vessels and causing thromboses and infarcts in the normal mouse.

Localization of Fungus Favored by Type of Inoculum.—In our experiments the aerial mycelium of the fungus has been ground in a mortar and inoculated. This method has probably resulted in more localized and encapsulated lesions than injections of suspensions of spores, because the ground whole mycelium contained fragments of brown hyphae and sporangia which have acted like inert foreign bodies. Probably the dosages employed would correspond to smaller dosages of pure suspensions of the spore forms.

Result of Inoculation of Suspensions of Spores.—In seven normal mice used as controls for an experiment on the effect of cortisone on the spread of Rhizopus, Spoto and Baker[11] found no extension of the fungus beyond the confines of the peritoneal cavity following intraperitoneal injections of R. oryzae. These mice were killed at intervals from 1 to 10 days. A few proliferating hyphae were found about the inoculum in the abdomen in one mouse, killed two days after inoculation. In another mouse, examined after 10 days, there were brown spores in two fibroblastic and neutrophilic nodular peritoneal scars. The explanation for the failure of spread in this series may be found in the smaller dosage employed: Only 0.2 cc. of a 3% suspension of spores, 1,700,000 spores, were injected intraperitoneally, whereas 1 cc. of a 10% suspension of the ground whole mycelium was injected into each of the mice in the present studies involving peritoneal infection.

Other Studies on Mucoraceae in Mice.—Christiansen in 1929 reported that he had inoculated white mice with species of Rhizopus and Absidia isolated from lesions of mucormycosis in swine.§ He obtained the same results from either of the two fungi. He found that white mice were very easily infected by injection into the veins or abdominal cavity of small amounts (0.1 cc.) of suspensions of spores. The animals died in about four days and showed renal changes only.

From a bovine lesion of mucormycosis, Davis (1955)[14] isolated a culture identified as Lichtheimia (Mucor) corymbifera. He writes:

> The fungus proved pathogenic for 9 of 10 mice by intravenous inoculation of the spores using either 0.1 ml. or 0.2 ml. of a saline suspension. Death occurred between 3 and 7 days with the 0.2 ml. dose and between 7 and 12 days with the 0.1 ml. injection. The fungus was recovered from the principal organs, but the kidney yielded the organisms most consistently. One mouse survived and was destroyed after 41 days, but a positive culture of Mucor was nevertheless made from its kidney. There were no discernible macroscopic lesions in the inoculated mice but, on microscopic examination of the various organs, only the kidneys showed significant lesions in the form of suppurative foci. The fungus was present within some of the inflammatory areas as well as within the renal pelvis.

These results are not comparable to ours, since injections were into veins instead of into the peritoneal cavity. Davis's results show the tendency of the Mucoraceae to involve the kidneys when the fungus is given intravenously, as was pointed out by Lichtheim in 1884 as characteristic of this route of inoculation in rabbits.[15]

A fungus derived from a spontaneous subcutaneous nodule of a mouse and identified as A. corymbifera (M. corymbifer) has recently been carefully studied in lesions of mice.[16] Suspensions of the spore forms of this fungus injected subcutaneously into mice produced a granulomatous lesion, which reached the peak of its proliferative period 16 to 25 weeks after inoculation when the

§ References 12 and 13.

nodule reached a diameter of 3 cm. Intraperitoneal inoculations of suspensions of 1000 and of 10,000 spores, which were small amounts of fungus in comparison with what we used, resulted in subcutaneous nodules along the injection route, and in two mice, a polypoid extension into the peritoneum, but no peritoneal or other lesions. The mice were killed 30 weeks after the inoculation, much later than any of the series of our mice inoculated with R. arrhizus.

The lesions caused by A. corymbifera, induced either by subcutaneous or intraperitoneal injection, appeared to be more localized than those of R. arrhizus, as no extensions to kidney, liver, spleen, or brain were observed. Spread to regional lymph nodes, however, occurred frequently.

Microscopically, the lesions caused by A. corymbifera presented hyphae with swellings and constrictions and even cyst-like appearances unlike the hyphae of R. arrhizus as seen in experimental lesions in mice or in the human cases of mucormycosis known to be caused by Rhizopus.‖ This suggests that Rhizopus is probably the causative agent of many of the recently reported cases of cerebral and pulmonary mucormycosis rather than Absidia (Mucor). The appearances of the fungus in tissues, together with cultural results, indicates that some of the cases of mucormycosis in animals and possibly some of the cases of mucormycosis in humans reported in the older German literature have been caused by fungi of the genus Mucor.[4]

SUMMARY

Intraperitoneal inoculations of 1 ml. of a 10% suspension of ground R. arrhizus fungus, obtained from a human case of mucormycosis, inoculated intraperitoneally into six normal mice resulted in peritoneal inflammatory masses. These masses consisted of the central inoculum with some proliferating hyphae surrounded by zones of polymorphonuclear leucocytes, macrophages, fibroblasts, and, later, collagenous connective tissue. These inflammatory masses were usually found in the left upper quadrant binding liver, spleen, pancreas, and stomach together. Some destruction of adjacent tissues with local invasion by the fungus occurred. These reactions were observed at various time intervals over a period of 37 days; and at least until 27 days the fungus could be recovered from the peritoneal cavity by culture on Sabouraud's agar. There was spread to liver and kidney in many cases, but no systemic dissemination of the fungus to thoracic or cranial organs.

Inoculation intraperitoneally of the same suspension into seven mice rendered diabetic with alloxan six to eight days prior to the inoculation resulted in tissue reactions up to 38 days similar to those in normal mice.

Intracerebral inoculations of 0.025 ml. of a 10% suspension of the mycelium and spores, with the killing of eight mice periodically over a period of 20 days, resulted in mild proliferation of the fungus with viable organisms recovered by culture as late as 20 days after inoculation. An inflammatory reaction was seen at the site of inoculation. The reaction was characterized by necrosis, polymorphonuclear and macrophagic response, and fibrous encapsulation. Meningitis, ependymitis, and inflammation of the choroid plexus were sometimes observed. There was no dissemination of the fungus beyond the brain and meninges.

Intracerebral inoculation of the suspension into six mice rendered diabetic by alloxan six to eight days prior to the inoculation resulted in features similar to those observed in normal mice.

It is concluded that alloxan-induced experimental diabetes, after it has been established for six to eight days, does not alter the reaction of mice to intraperitoneal or intracerebral inoculations of R. arrhizus. This is in contrast to the increased susceptibility to spread of the fungus in the acutely toxic stage of alloxan diabetes in rabbits. Hyperglycemia in itself, therefore, does not appear to increase the host susceptibility of the mouse to this experimental infection.

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Myelolipoma of Adrenal

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The adrenal gland continues to assume ever-increasing importance in all fields of medicine and surgery. The isolation, identification, and synthesis of the various crystalline adrenocortical steroids have brought new understanding of the nature and treatment of a multitude of disorders previously thought to be unrelated. The availability of these hormones as replacement therapy has also given great stimulus to adrenal surgery in the fields of neoplasia, hypertension, etc. With all these advances in knowledge of the role of the adrenal in disease, it is unfortunate that the search for the presence of corresponding morphologic change has not been as fruitful.

As experience in the pathologic anatomy of the adrenal increases, one wonders if some of the minute but fairly common findings in the adrenal at autopsy which are now sometimes considered coincidental and unimportant will assume much broader significance.

One such finding is the basis for this report. It is the presence of varying-sized areas containing fat and hemopoietic tissue in the cortex of the adrenal gland. Although receiving scant attention in recent medical literature, approximately 27 cases occurring in humans have been reported in the last 25 years.

In 1932 Collins [1] reviewed the literature and found 15 cases, to which he added 1. Almost all of the cases were from the European literature. Among these was a report by Arnold in 1866, which was apparently the first recorded case. The patients whose ages were given varied between 42 and 75 years, with the average being 60. Collins' personal case was that of a 32-year-old man. The average size of the lesions in this series was 1.5×0.9 cm. Four patients in the series had advanced cardiovascular disease; two had malignancy.

Only sporadic reports of this finding have appeared since. The case published in 1935 by DeNavasquez [2] was that of a 39-year-old man, and the lesion was considered unique because of its size. It measured 8×6.6×7.4 cm. and caused the adrenal to weigh 240 gm. Also in 1935, Richardson [3] reported the occurrence in a 37-year-old woman of a bone-marrow-containing tumor, which measured 8×5.5×3 cm. Giffen,[4] in 1947, reviewed the literature and added seven cases.

In a consecutive series of 2000 autopsies done in the Jefferson Medical College Hospital over a six-year period, the diagnosis of myelolipoma of the adrenal gland was made four times, an incidence of 0.2%. A brief description of these cases and the adrenal findings follow. Because the present status of our understanding of the lesion under discussion precludes correlating it with the sequential development of any disease, no attempt will be made to present the clinical histories or postmortem findings in any detail. Attention will be directed only to the adrenals in these cases.

REPORT OF CASES

CASE 1.—The patient was a 71-year-old white man who died approximately six months after a diagnosis of prostatic cancer was made. A limited autopsy revealed adenocarcinoma of the prostate with metastasis to the liver and adrenals. The adrenals were slightly enlarged and cut with ease. The cortex, bilaterally, revealed increased pigmentation and contained multiple nodules measuring up to 1 cm. in diameter. The nodules varied from

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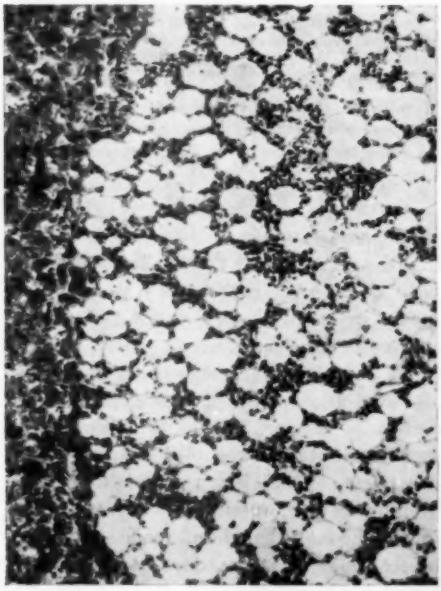
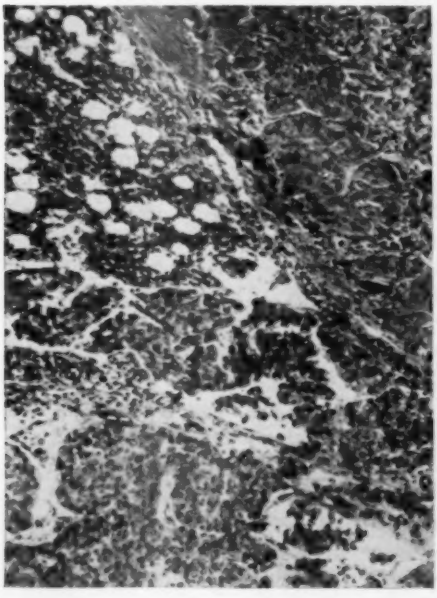


Fig. 1.—Adrenal containing nodule of adipose tissue and primitive cells of myeloid and erythroid series in a proportion suggestive of bone marrow.

Fig. 2.—Adrenal showing intimate association of myelolipoma (upper left and center) and metastatic carcinoma (lower left).



grayish-pink in color and were well-circumscribed. Histologically, these nodules were seen to be composed of adipose tissue containing foci of hemopoiesis (Fig. 1). The relative amounts of each component varied from nodule to nodule and occasionally within the same nodule. In addition, there were occasional irregular, non-nodular collections of the same type of tissue, which seemed to have insinuated themselves between the cords of adrenal cells. This latter finding has, as far as can be determined, not been mentioned in the cases previously reported in the literature. Another interesting feature in this case was the intimate association in one area of metastatic tumor and myelolipoma (Fig. 2).

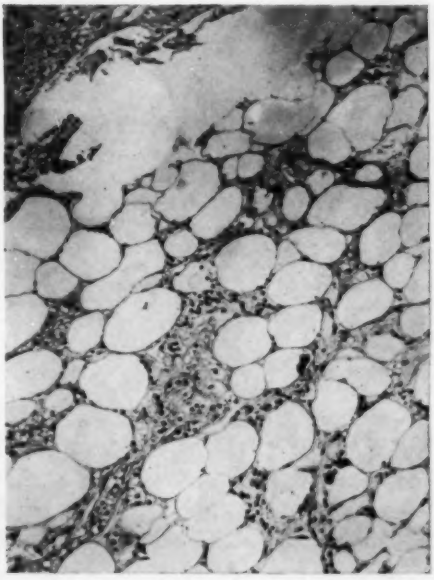


Fig. 3.—Adrenal with nodule of adipose tissue and only sparse amount of hemopoiesis.

Case 2.—The patient was a 58-year-old white woman who died on the seventh hospital day with severe uncontrolled diabetes complicated by pneumonia and subarachnoid hemorrhage. One year prior to death she had been treated with radioactive iodine for a toxic goiter, with a subsequent alleviation of her hyperthyroidism. The adrenals, grossly, were remarkable only in that the left contained a well-circumscribed, yellowish, cortical nodule which was macroscopically interpreted as a cortical adenoma. Histologic examinations substantiated this impression but, in addition, there was an irregular, microscopic focus of adipose tissue and hemopoiesis. The myeloid and erythroid elements were sparse in

this case, the predominant feature being the fat (Fig. 3).

CASE 3.—The patient was a 22-year-old white woman, admitted to Jefferson Hospital with neurological symptoms and signs of about seven months' duration. She died approximately 24 hours after craniotomy. Autopsy substantiated the clinical diagnosis of Arnold-Chiari deformity (platybasia). The adrenals on external examination were not remarkable, but the right, on section, contained scattered focal calcified areas. Histologically there were seen nodules composed predominantly of fat but also containing areas of hemopoiesis. In addition, the hemopoietic foci were in association with focal

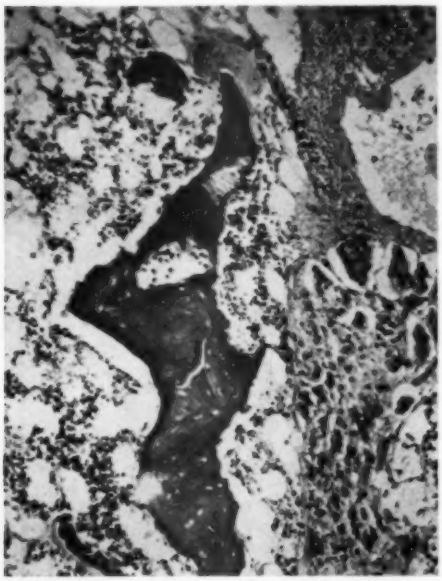


Fig. 4.—Adrenal with nodule of myelolipoma containing recognizable bony trabeculae.

areas of calcification and occasionally with well-developed bony trabeculae (Fig. 4).

CASE 4.—The patient was a 50-year-old white woman, admitted to Jefferson Hospital complaining of dyspnea, orthopnea, and severe chest pain. She had been a known hypertensive for 15 years and had had a bilateral sympathectomy 4 years before this admission. The patient followed a generally downhill course and died suddenly, six weeks after admission. Prior electrocardiographic studies had revealed an acute anterior myocardial infarction. Autopsy revealed severe coronary arteriosclerosis with recent and old myocardial infarctions.

The left adrenal contained a nodule having the same golden-yellow color as the cortex. It measured 0.75 cm. in its widest diameter. Histologically the nodule was seen to be made up of fat intermixed with adrenocortical-like cells distinguished from those of the remaining cortex by their increased size and lipoidal content. Only a rare cluster of primitive myeloid and erythroid elements was seen. The nodule was sharply demarcated from the surrounding adrenal tissue but was not encapsulated.

COMMENT

The observation made by previous authors that the relative amounts of hemopoietic and fatty tissue vary considerably is borne out in our cases. In Cases 2 and 4 the myeloid and erythroid elements were sparse, whereas in Case 3 they were present in abundance and were also associated with well-developed trabeculae of bone. This latter finding would seem to represent the ultimate in the development of the lesion under discussion. Mention of the presence of bony trabeculae in the nodules has been made only once before in the literature, one of Giffen's cases possessing this feature.

There is a great deal of uncertainty concerning the etiologic basis for myelolipoma. The older German literature referred to by Collins and Giffen brought forth a number of hypotheses, among which were ectopia, embryonic cell rests, developmental malformations, and metaplasia of adrenocortical cells. DeNavasquez thought it unnecessary to invoke these aforementioned theories. He preferred to consider the presence of blood-forming tissue in the adrenal simply as a manifestation of the potential hemopoietic ability possessed by this tissue because of its mesenchymal origin. Although not believing that this is a truly neoplastic lesion, DeNavasquez accepted the term myelolipoma, which he states was first used by Oberling in 1929. This has become the most widely used designation for the lesion, undoubtedly because it is descriptive without specifying etiology.

Probably the most interesting recent consideration of this lesion was by Selye and Stone [6] in 1950. They had repeatedly noticed the appearance of islets of lymphocytes and polymorphonuclear leucocytes in the adrenals of many of their tumor-bearing rats, a finding also noted by Lewis [6] in 1937. As an

outgrowth of these observations they determined that the corticotropic action of the anterior pituitary in the presence of toxic products of tissue decomposition stimulated hemopoiesis of the adrenal gland of the rat. They further showed that the injection of methyltestosterone caused the appearance of definite fat cells in the adrenocortical tissue of rats. They then exposed animals to a combination of both of these substances and were able to produce hemopoiesis in a fatty stroma indistinguishable from normal bone marrow. It would seem then that there was artificially produced in these animals a situation in the adrenal gland quite like myelolipoma.

The cases herein described have little in common to which a possible etiologic basis for the adrenal findings might be ascribed. Their ages ranged from 22 to 71, with an average of 50. The predominant underlying lesions were adenocarcinoma of the prostate, diabetes mellitus and hyperthyroidism, Arnold-Chiari deformity, and hypertensive cardiovascular disease with arteriosclerosis. This is somewhat in keeping with Selye and Stone's notation that a large number of previously reported myelolipomas of the adrenal were in patients suffering from malignant tumors and cardiovascular disease. In connection with our one case with a history of hyperthyroidism, it is also worthy of note that Selye and Stone used thyroxin to augment their experimental production of bone marrow in the adrenal by the injection of testosterone and anterior pituitary extract.

SUMMARY

In a consecutive series of 2000 autopsies done over a six-year period in the Jefferson Medical College Hospital the diagnosis of myelolipoma of the adrenal gland was made four times, an incidence of 0.2%. No clinicopathological correlation is possible, except that a previously mentioned association of myelolipoma of the adrenal with malignant tumors and cardiovascular disease is noted in two of the four cases.

A brief review of the literature reveals 27 cases previously described. The various ideas as to etiology are referred to briefly.

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Transmission of the Common Cold Virus Strain MR to Suckling Hamsters

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The common cold (acute coryza, acute rhinitis) is attributable to infection of the upper respiratory tract with a virus. The symptoms and signs indicate the presence of an acute but transient alteration in the physiology of the mucous membrane of the upper respiratory tract, particularly that lining the nose and the paranasal sinuses. Dochez and co-workers [1] first reported successful transmission of the common cold to chimpanzees by means of filtrates. Many research workers,* ourselves included, have attempted to introduce the cold virus into a number of mammals, and all results were negative. The purpose of the experiment reported here was to try suckling animals that could be obtained easily and economically, if they were found to be susceptible, and suckling hamsters met these criteria for our trials. Six-day-old suckling hamsters with the mother were obtained from a reliable dealer in Silver Spring, Md. The dealer stated that his hamster colony was in excellent condition.

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* References 2 and 3.

MATERIALS

Saline washings of the nose and throat were obtained from a young woman two days after the onset of a typical common cold. The clinical picture was one of typical nasopharyngitis with a slight soreness or roughness of the throat. The nasal and nasopharyngeal mucosa was swollen and injected. The patient's temperature remained normal. For the normal control, saline washings were obtained from the nose and throat of a worker helping in the experiment, with no history of a cold for the past year and in excellent general health.

EXPERIMENTAL PROCEDURE AND RESULTS

The infected nasal and throat washings from the patient (strain MR) and the normal nasal and throat washings from the control were each filtered through a type ST size L3 Seitz filter. The filtrates were then used to initiate the following experiment.

Four lactating hamsters and their 6-day-old sucklings were placed in individual metal boxes with bedding. The suckling hamsters (Groups 1 and 2) were given the cold virus suspension (1 minim for both nostrils) by intranasal instillation. Groups 3 and 4 were treated likewise, with use of the normal nose and throat washings, as shown in the Table. Three days after exposure to the cold virus suspension, all sucklings (8 of 11) showed cold virus signs such as running nose, and wheezing, and the nostril area was swollen, boggy, and inflamed. The suckling hamsters from Groups 3 and 4 (normal control) showed no cold symptoms. Nose and throat washings made with sterile isotonic saline were obtained from Groups 1 and 2 suckling

hamsters, pooled and instilled nasally into Groups 5 and 6 sucklings. Three days later almost all suckling hamsters from these groups (10 out of 12) showed the symptoms described above. The virus was carried up to and including four passages in suckling hamsters, as shown in the Table. (Passages are being carried at the present time.) The infective cold virus material from the fourth hamster passage was instilled nasally into hamsters having recovered from the cold virus and into hamsters having no symptoms of a cold. The immune animals showed no symptoms, whereas the nonimmune ones developed a typical cold after three to five days.

Cold Virus (Strain MR) Passages in Suckling Hamsters

Group No.	No. Exposed	No. Showing Cold Signs	No. Showing No Cold Signs	Onset of Disease, Days	Passage No.
1	11	8	3	4	1
2					
5	12	10	2	4	2
6					
7	11	11	0	3	3
8					
9	14	10	4	3	4
10					
3	12	0	12	..	..
4 (normal control)					

SUMMARY

From the data obtained in this study, it appears that this MR cold virus strain was transmitted to suckling hamsters. This is the first time that a cold virus strain has been transmitted to an animal other than the chimpanzee. The cold virus from the fourth suckling hamster passage was confirmed to be cold virus after tests conducted with immune and nonimmune hamsters.

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Experimental Histoplasmosis in Immunized and Nonimmunized Mice

Comparative Pathology

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and
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The problem of therapy of histoplasmosis is unsolved. Although most infections with Histoplasma capsulatum are benign, the number of reported fatal cases is increasing.* Recently, the importance of chronic pulmonary histoplasmosis has been indicated.† The frequent infection of laboratory personnel working with the fungus has been well documented.‡ Since no efficient drug therapy is available, studies of immunization against this disease would seem timely. Exposed laboratory personnel and other selected persons might be candidates for such prophylaxis. Evidence has been presented that a positive histoplasmin skin test indicates increased resistance to disease.[9] No cases of repeated infections in laboratory personnel have occurred.

Repeated skin tests with histoplasmin have been reported to stimulate circulating antibody as measured by soluble antigens § but not by whole yeast-like cell as antigen.[12] However, the relationship between these circulating antibodies and immunity, if any exists, has not been determined.

In his studies of ocular histoplasmosis, Day [13] demonstrated a modification of the course of infection in the remaining eye of rabbits which had had one eye inoculated with the mycelial phase of H. capsulatum 6 to 14 weeks previously. This immune effect was not demonstrable if the interval between infections was only two to four weeks.

Protection of mice against lethal infections by prior inoculation of dead or live cells of the yeast phase has been demonstrated.‖ Quantitative cultural studies showed that this immunization tended to lower the number of fungus organisms in the tissues rather than to eliminate them. This effect, however, was usually sufficient to prevent death in the protected mouse subjected to lethal challenge. Some of these results have been confirmed by Schaefer and Saslaw.[17] Farrell and coworkers [18] presented evidence suggestive of immunity in dogs following a sublethal intratracheal inoculation.

The purpose of this study of immunity to H. capsulatum was to observe the pathologic changes in both immunized and nonimmunized mice. In this paper, pathologic changes observed in tissues of both groups

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* References 1 and 2.

† References 3, 4, and 5.

‡ References 2 and 6-8.

§ References 10-12.

‖ References 14-16.

are described, and differences in the extent of lesions are associated with the immune response.

MATERIALS AND METHODS

Fungus.—The yeast phase of H. capsulatum isolated No. 6515 was used throughout the study. It was maintained by growth at 37 C on an agar medium of 51 gm. per liter of Difco cystine-heart agar and 20 gm. per liter of Difco Bactohemoglobin. The yeast-phase cells employed for animal inoculation were grown for 48 to 72 hours at 37 C under constant rotation in a liquid medium.[14]

The antigen employed for immunization was prepared by growing cells of the yeast phase at 37 C in the foregoing medium for five days under constant rotation. The yeast-like cells were then killed by exposure to 0.5% formalin for 72 hours, washed thoroughly in 0.85% saline, and subsequently dried in acetone at —10 C.

Animals.—Male white Swiss mice of the Rocky Mountain Laboratory strain were employed. They were 21 days old at the beginning of each experiment.

Cultures.—Quantitative cultures of tissue were performed on pools of liver, spleen, brain, or lung from three mice. The tissue was removed aseptically, immediately weighed, and ground thoroughly in sterile sand. A 10% suspension by weight was made in sterile 0.85% saline. Serial decimal dilutions were made in saline, and 0.1 ml. of each dilution inoculated in duplicate on Sabouraud's agar (4% dextrose, 1% Difco neopeptone, adjusted to a final pH of 5.9).[15] All cultures were incubated at room temperature for 28 days before results were recorded.

Pathology.—Microscopic studies were made on a variety of organs. These tissues were fixed in 10% Zenker-formal solution. The lungs were fixed by intratracheal injection of this solution. The tissues were sectioned by the paraffin method and stained with hematoxylin and eosin.

When cultural studies were performed, the liver, spleen, and brain were divided and microscopic sections prepared from each specimen. Owing to the method of fixation, half-portions of the lungs could not be cultured. Therefore, only three or four lung specimens at each time interval were available for microscopic study.

Immunization and Challenge.—In four experiments immunization was attempted by intraperitoneal injection of 5 mg. of the killed acetone-dried antigen in three or four divided weekly doses. In a fifth experiment protection was sought by prior sublethal intraperitoneal infection. Mice were challenged in the first four experiments with a sublethal inoculum by the intracerebral or intranasal route. The mice in the fifth experiment (except for one control group) were challenged with a lethal intracerebral dose. The details of these experiments as to immunization schedule, route and size of challenge dose, and killing schedule for cultural and histologic examination are summarized in Table 1.

TABLE 1.—*Immunization Schedule, Challenge Route and Dosage, and Time Intervals of Killing for Culture and Pathology in Five Experiments*

		Immunization Schedule			Challenge			Killing Schedule	
Experiment	Group	Total Dose	No. of Equally Divided Doses	Time Interval Between Doses, Days*	Route	Mouse Age at Challenge, Wk.	No. of Yeast-like Cells	No. Examined at Each Time Interval	Time Intervals After Challenge
1	A, immunized	5 mg., inactivated	3	7	I. C.	6	10^5	3	1, 2, 4, 7, 10, 14, 18 days
	B, control	None	..	..	I. C.	6	10^5	3	1, 2, 4, 7, 10, 14, 18 days
2	A, immunized	5 mg., inactivated	4	7	I. C.	7	10^5	9	4, 7, 14 days; 1, 2, 4 mo.
	B, control	None	..	..	I. C.	7	10^5	9	4, 7, 14 days; 1, 2, 4 mo.
3	A, immunized	5 mg., inactivated	3	7	I. N.	6	10^5	4	1, 2, 3, 5, 7, 9 wk.
	B, control	None	..	..	I. N.	6	10^5	4	1, 2, 3, 5, 7, 9 wk.
4	A, immunized	5 mg., inactivated	4	7	I. N.	7	10^5	12	2, 4, 7, 14 days; 1, 2, 4 mo.
	B, control	None	..	..	I. N.	7	10^5	12	2, 4, 7, 14 days; 1, 2, 4 mo.
5	A, immunized, not challenged	10^6 live	1	..	None	..	....	3	4 hr., 1, 2, 3, 4, 7 days
	B, immunized	10^6 live	1	14	I. C.	5	4×10^7	3	4 hr., 1, 2, 3, 4, 7 days
	C, control	None	..	..	I. C.	5	4×10^7	3	4 hr., 1, 2, 3, 4, 7 days

* All mice were three weeks old at onset of immunization schedule.

RESULTS

A. Intracerebral Infection Following Immunization with Formalin-Inactivated Cells of Yeast-Phase Histoplasma

In Experiments 1 and 2 less extensive changes were observed microscopically in the tissues of immunized mice than in the tissues of control mice. This difference was most marked in the brains. In Experiment 1 there was nearly complete sparing found in the brains of the immunized animals. Despite the lack of disease in the brain, the immunized mice in this experiment showed the same type of focal granulomas in the liver as were present in the liver of the controls, although in somewhat fewer numbers. Experiment 2 showed similar sparing following immunization, but not as uniformly and to a somewhat slighter extent.

A detailed description of the pathology observed in these two experiments follows.

There were no consistent gross pathologic changes.

Brain.—Changes were first observed in the brains of the control mice two days after inoculation. An inflammatory cell infiltrate, with some edema and cellular exudate, was seen in the meninges. Lymphocytes were the predominating cell type in this infiltrate, although polymorphonuclear leucocytes were present in irregular numbers. Cells of H. capsulatum could usually be found, but the numbers easily seen varied from specimen to specimen. The intensity of the reaction and the amount of exudate increased through the fourth day after inoculation (Fig. 1*B*), and sometimes through the seventh day (Fig. 1*D*). The most extensive acute meningitis was observed in some of the seven-day specimens. On the other hand, beginning of clearing with decrease in the amount of exudate and disappearance of neutrophiles and fungus cells was noted in many seven-day specimens. Occasionally at seven days after challenge in specimens with extensive meningitis, parenchymal involvement adjacent to the meninges was noted. By 14 days the exudate had disappeared and the predominant lesion was thickened adherent meninges. Occasional foci of round cells were noted in the brain substance.

In Experiment 1, two of the brains from immunized mice four days after challenge showed an occasional macrophage with fungus cells in the meninges but with only minimal inflammatory cell infiltration (Fig. 1*A*). The rest of the brains of the immunized mice in this experiment examined over the 18 postchallenge days were entirely normal.

In Experiment 2, there was again a marked difference in the amount of disease observed in the brains of immunized mice. The majority of these mice had a meningeal reaction definitely of less extent than the controls. However, during the first two weeks after challenge a few of the brains from the immunized group had as extensive a meningitis as the controls, whereas others showed no disease at all (Fig. 1*C*).

All of the brains from immunized mice and somewhat less than half of the brains of the control mice showed no abnormalities at the one-, two-, and four-month periods. In the brains from control animals with disease, parenchymal abscesses were regularly present, with an occasional specimen showing only thick adherent meninges (Fig. 1*E* and *F*). The extent and number of these abscesses varied among the specimens. The abscesses typically had necrotic centers surrounded by areas with round cells and macrophages. Cells of the yeast phase of H. capsulatum were usually abundant both in macrophages and in extracellular debris. Most of the abscesses were quite well localized and frequently located near a meningeal surface.

Liver.—An occasional small collection of round cells was observed in a few of the livers examined two days after inoculation. By four days all the livers examined showed lesions. These varied in size from very small up to about 100μ, and tended to be localized and rounded. Some consisted merely of collections of lymphocytes, while some of the larger lesions appeared to show necrotic liver parenchymal cells surrounded by round cells (Fig. 2*A*). Scattered lymphocytes were occasionally seen. The lesions were numer-

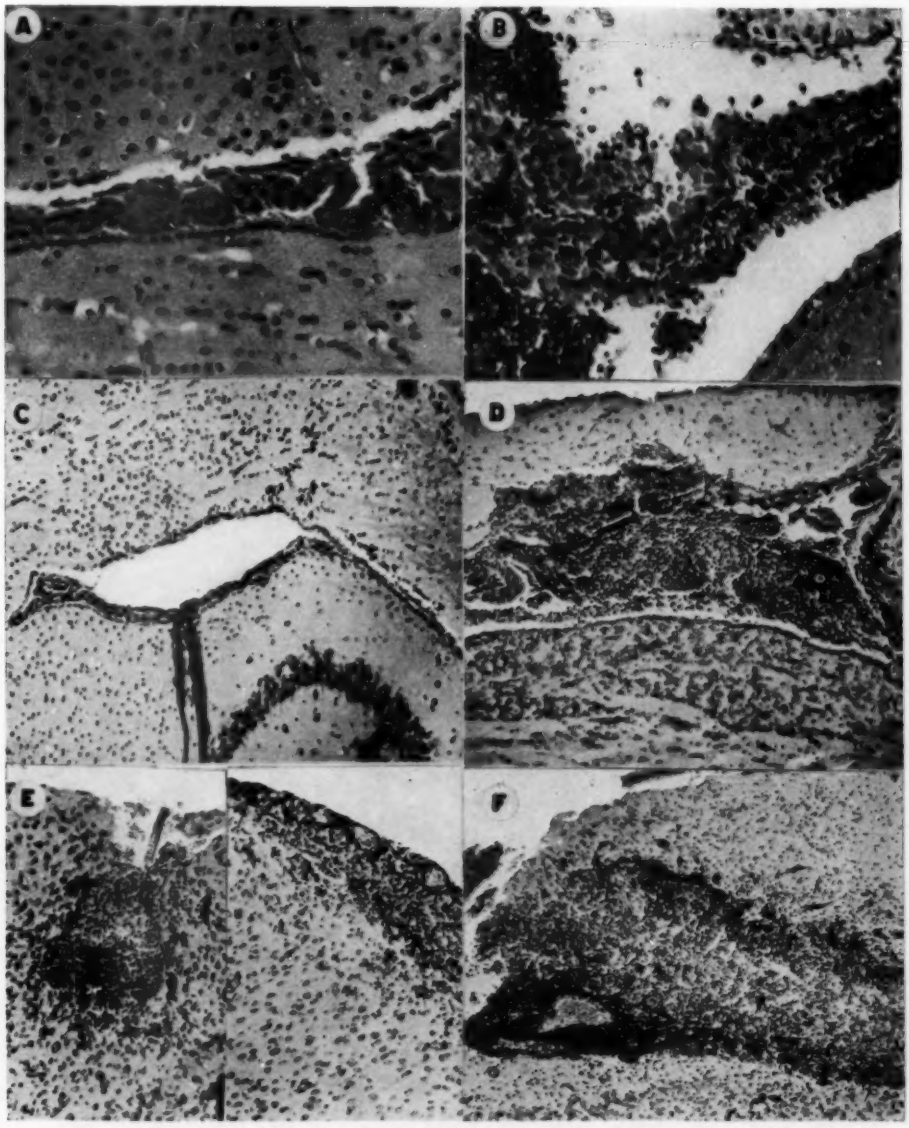

Fig. 1.—Photomicrographs of brain sections from mice immunized with inactivated cells of the yeast phase of H. capsulatum (see text) and from control mice, both groups being challenged intracerebrally with 10^3 live yeast-phase organisms. All sections stained with hematoxylin and eosin.

A (Experiment 1), brain of immunized mouse four days after challenge, showing occasional macrophage filled with fungus cells but with only minimal inflammatory-cell infiltration present in the meninges; reduced about 1/5 from mag. × 300.

B (Experiment 1), brain of a control mouse four days after challenge, showing considerable meningeal infiltration, predominantly lymphocytic; reduced about 1/5 from mag. × 300.

C (Experiment 2), brain of immunized mouse seven days after challenge, with normal-appearing meninges; reduced about 1/5 from mag. × 120.

D (Experiment 2), brain of a control mouse seven days after challenge, having extensive meningitis with infiltration of polymorphonuclear leucocytes as well as lymphocytes; reduced about 1/5 from mag. × 120.

E (Experiment 2), two areas from brain of a control mouse 28 days after challenge, showing a small parenchymal abscess and thickened adherent meninges; reduced about 1/5 from mag. × 120.

F (Experiment 2), brain of another 28-day control mouse with a large parenchymal abscess; reduced about 1/5 from mag. × 90.

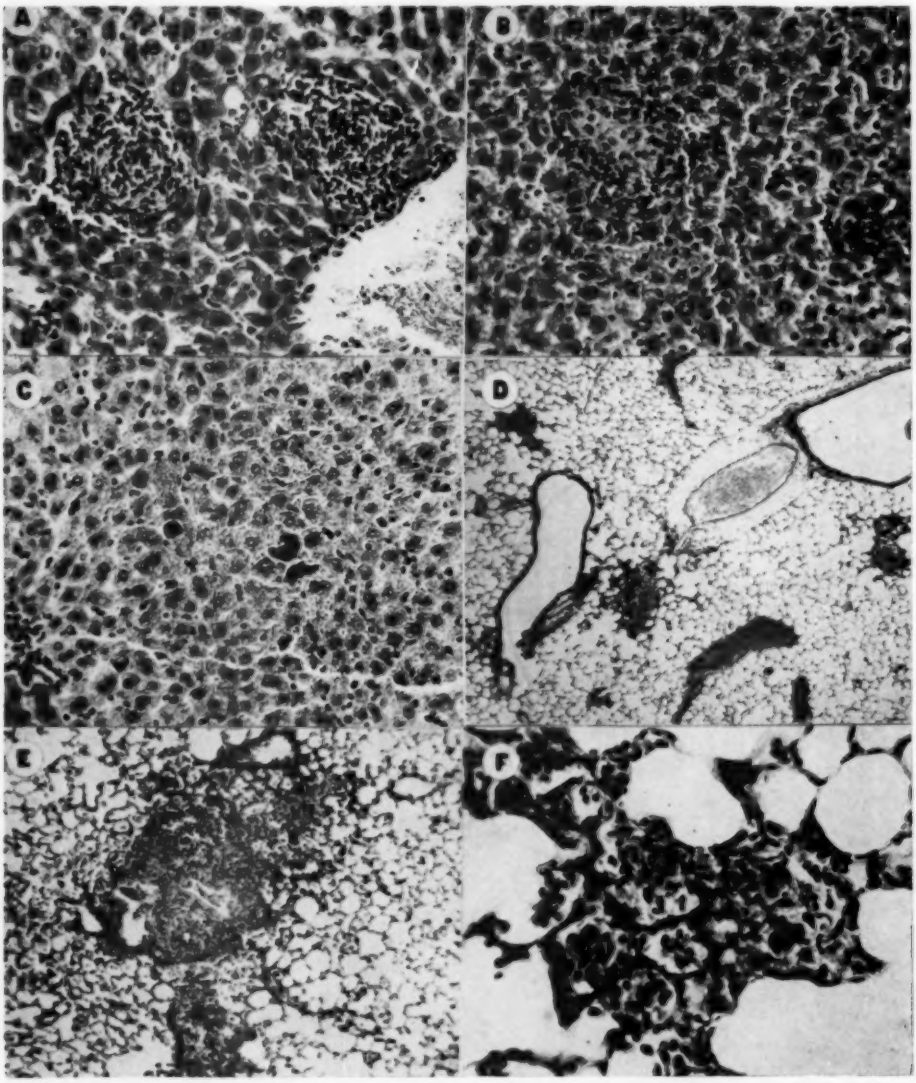

Fig. 2.—Photomicrographs of liver and of lung sections from mice immunized with inactivated cells of the yeast phase of H. capsulatum (see text) and from control mice after challenge intracerebrally (liver sections) with 10^5 live organisms or intranasally (lung sections) with 10^6 live yeast-like cells. All sections stained with hematoxylin and eosin.

A (Experiment 2), two early granulomas in the liver of an immunized mouse four days after challenge; reduced about 1/5 from mag. × 250.

B (Experiment 2), mature epithelioid granuloma in the liver of an immunized mouse seven days after challenge; reduced about 1/5 from mag. × 250.

C (Experiment 2), end-stage of healing granulomas just prior to complete resolution in the liver of an immunized mouse two months after challenge; reduced about 1/5 from mag. × 250.

D (Experiment 4), three small areas of infiltration in the lung of an immunized mouse seven days after intranasal challenge; reduced about 1/5 from mag. × 45.

E (Experiment 4), peribronchial location of lesions in the lung of a control mouse seven days after challenge; reduced about 1/5 from mag. × 80.

F, high-power view of *D*, illustrating the predominantly round-cell type of infiltration and the intact alveolar walls; reduced about 1/5 from mag. × 375.

ous in most specimens. There was a definite though slight difference between the immunized and control groups, with more lesions being present in the controls.

By seven days even more numerous lesions were generally observed. The necrotic centers of the lesions were no longer prevalent, but various-sized round lesions made up mostly of lymphocytes and resembling early granulomas were scattered throughout the parenchyma. Some of the larger lesions (about 75μ) showed epithelioid cells in the center and definite swirling of the surrounding lymphocytes. These maturer granulomas were few in number, and they were usually seen in the immunized mice (Fig. 2*B*). Yeast-like cells of H. capsulatum could occasionally be demonstrated in small numbers in these lesions, but only after careful search.

By 10 and 14 days after challenge many more of the liver lesions showed epithelioid cell centers and thinner layers of surrounding lymphocytes. However, numerous lesions in various stages of maturity were present in all livers at this time. In those specimens obtained one month after challenge some entirely normal-appearing livers were seen in both the control and the immunized groups. The proportion of normal livers and the scarcity of lesions in the others increased in the two- and four-month specimens. In some of the otherwise normal livers perivascular cuffing by lymphatic tissue was present. This cuffing was noted particularly in the immunized mice. In the livers still containing lesions mature epithelioid granulomas were present. Small irregular collections of deeply basophilic cells coalescing into giant cells were present, apparently representing the end-stage of the lesion prior to complete resolution (Fig. 2*C*). Histologic evidence of active liver disease was present in only a few mice killed one to four months after challenge. These lesions were all in control mice and were present in mice whose brains had extensive active abscesses. In these livers, in addition to numerous scattered granulomas in various stages of maturity, diffusely scattered lymphocytes and, even less commonly, large areas containing considerable inflammatory infiltrate and numerous yeast bodies of H. capsulatum were present.

Spleen.—Despite the fact that numerous viable organisms of H. capsulatum were usually cultured from the spleens (see below), only rarely was it possible to demonstrate microscopic changes in this organ. Hyperplasia could occasionally be demonstrated in specimens later than seven days after challenge. At one month after challenge, a spleen from an animal with severe chronic brain disease and active liver disease contained several small granulomatous structures. A few organisms of the yeast phase were found in these lesions.

Other Organs.—Abnormalities that could be associated with histoplasmosis infection were not found in sections of kidney, adrenal, intestine, pancreas, or lung.

Cultures. — Quantitative cultures were made on organs in Experiment 2. These tend to support the microscopic findings (Table 2).

TABLE 2.—*Comparison of the Numbers of Viable Cells or Cell Aggregates of H. Capsulatum Cultured from Ground Pooled Tissues of Immunized and Control Mice at Specific Intervals After Intracerebral Challenge with 10^8 Cells of the Yeast Phase—Experiment 2*

Time After Challenge	Log of No. of Fungus Cells per 0.01 Gm. of Tissue					
	Immunized Mice*			Control Mice		
	Liver	Spleen	Brain	Liver	Spleen	Brain
4 days	4†	6	4	5	6	4
	4	5	4	4	>6	4
	3	4	4	4	6	3
7 days	6	5	>6	5	>6	>6
	5	6	4	4	>6	>6
	4	6	4	5	5	>6
14 days	3	4	4	5	>6	>6
	2	5	3	4	5	5
	3	3	3	3	4	>6
28 days	2	4	1	4	>6	>6
	1	3	2	3	4	3
	1	3	1	N. D.‡	..	..
2 mo.	2	3	2	2	3	>6
	2	3	0	0	3	5
	2	2	0	1	4	4
4 mo.	1	0	0	>6	6	5
	0	1	0	2	3	4
	0	0	0	0	1	1

* See text for details of immunization.
† Each value is for a pool of three mice. Three such pools were cultured at each time interval.
‡ N. D., not done.

There were definitely fewer viable cells of H. capsulatum in the tissues of the immunized mice than in those of the control mice. This difference occurred in all three of the tissues examined, although it was more marked in the brains than in the livers and spleens. As time after challenge elapsed, there was a consistent steady decrease in the numbers of fungi cultured from the tissues of the immune mice. Irregular results were observed in the control mice, with some high titer pools at the later time intervals.

B. Intranasal Infection Following Immunization with Formalin-Inactivated Cells of the Yeast Phase

Microscopic examination of the several organs revealed no demonstrable differences between the immunized and control mice following sublethal intranasal challenge (Experiments 3 and 4). The absence of histopathologic differences between the two groups is supported by the quantitative cultural results, which also failed to show differences between the two groups (Table 3).

Table 3.—*Comparison of the Numbers of Viable Cells or Cell Aggregates of H. Capsulatum Cultured from Ground Pooled Tissues of Immunized and Control Mice at Specific Intervals After Intranasal Challenge with 10^8 Cells of the Yeast Phase—Experiment 4*

Time After Challenge	Log of No. of Fungus Cells per 0.01 Gm. of Tissue					
	Immunized Mice*			Control Mice		
	Liver	Spleen	Lung	Liver	Spleen	Lung
2 days	0†	0	6	0	0	5
	0	0	4	0	0	5
	C‡	C	C	0	0	0
4 days	3	1	6	2	2	6
	2	2	C	3	2	5
	1	1	6	1	1	4
7 days	4	4	>6	4	5	>6
	4	3	5	3	3	>6
	3	4	0	C	C	C
14 days	>6	>6	>6	>6	6	>6
	5	6	C	6	5	>6
	3	3	C	4	4	>6
28 days	4	3	>6	3	4	C
	2	3	C	2	4	2
	C	C	C	1	2	0
2 mo.	0	4	4	1	2	1
	C	2	0	1	2	C
	0	0	0	1	2	0
4 mo.	0	0	0	0	0	0
	0	0	0	0	0	0

* See text for details of immunization.

† Each value is for a pool of three mice. Three such pools were cultured at each time interval except four months.

‡ C, contaminated.

The pathologic changes in the mice of Experiment 3 with the smaller challenge dose were minimal and irregular. Many lung sections were normal. Small collections of round cells including occasional macrophages were observed peribronchially in some specimens. More consistently, focal round-cell collections were found in the livers. Mature granulomas did not develop, and these lesions as well as the lung lesions appeared to be resolving in the five- and seven-week specimens. The disease which resulted from a still sublethal but larger challenge dose used in Experiment 4 was much more extensive and provided the material for the following description of the development of pathologic changes after intranasal inoculation.

There were no consistent gross pathologic changes.

Lungs.—In the lungs peribronchial infiltration could be seen in some specimens at the first examination period two days after challenge. These lesions were small, involving only a few alveoli, and they consisted of intra-alveolar infiltrates of lymphocytes and macrophages. There was no edema and very few polymorphonuclear leucocytes. At four and seven days the presence of the peribronchial lesions was more consistent (Fig. 2*D*, *E*, *F*), and the lesions tended to be slightly larger. The involved alveoli appeared to be rather loosely packed with round cells. Histoplasma organisms usually were present intracellularly in macrophages. In both the immunized and control groups there was an occasional lung without lesions. This resulted most likely from the lack of uniformity of dosage inherent in the method of intranasal infection. There was also an occasional lung with a larger lesion than the typical ones described above. These larger lesions usually showed some atelectasis and did not differ in cell type.

The lesions were sharply localized by 14 days, and thereafter they healed by resolution. The development of epithelioid granu-

loma previously observed in rabbit lungs ¶ after air-borne infection with H. capsulatum spores was not found in these experiments. At two and four months after challenge only occasional resolving parenchymal lesions were still present, although the lungs frequently contained unusual amounts of lymph tissue, particularly around blood vessels. In several mice secondary bacterial invasion of the lung lesions occurred. This is not unusual following the intranasal instillation of fluid. A bronchopneumonia picture is produced with numerous polymorphonuclear leucocytes throughout the parenchymal lesions and with a purulent bronchial exudate.

Liver and Spleen.—The disease in the livers and spleens was found to be similar to that reported above for the intracerebrally challenged mice. No differences between the immunized and the control mice could be found. In the livers, round-cell lesions appeared by the fourth day after challenge. These lesions developed into progressively maturer granulomas, with the height of disease at 14 days, and then gradually healed by resolution thereafter. Considerable variation from specimen to specimen in the amount of disease was observed after the intranasal challenge. A few of the liver specimens demonstrated as extensive disease as in any of the intracerebrally challenged mice. Only rarely was any abnormality found in the spleens.

Other Organs.—No abnormalities attributable to histoplasmosis could be found in kidney, adrenal, intestine, pancreas, or brain.

C. Lethal Intracerebral Challenge Following Sublethal Intraperitoneal Infection

In Experiment 5 the favorable influence of sublethal infection on subsequent lethal challenge was most obvious in the mortality results. While all 200 mice of control Group C died within two weeks of the intracerebral inoculation with 4×10^7 yeast cells, only 48 of 92 previously infected Group B mice succumbed to the same challenge. In the 100 Group A animals which received only the sublethal intraperitoneal infection, no deaths occurred during one month of observation.

The effect of the sublethal infection on subsequent lethal challenge was indicated microscopically by a decrease in the amount of disease observed in the brains of Group B mice as compared with the brains of Group C mice. Meningitis was present one day after intracerebral challenge in both these groups (Fig. 3*C*). The amount of disease in the Group C control mice became extensive by two days after challenge, and by the third day after challenge parenchymal abscesses were present in the brains. Mice killed later in the week uniformly had extensive pathologic changes with abundant polymorphonuclear leucocytes and cells of H. capsulatum in both the meninges and the brain parenchyma (Fig. 3*E*). In the Group B mice the development of disease was retarded, and the uniformity of findings seen in the control animals did not exist. About one-third of the brains examined showed no disease, while a few others showed only minimal meningitis (Fig. 3*B*). Many of the brains showed a moderately extensive meningitis, with parenchymal lesions observed in some of the four- and seven-day specimens. The nature of the lesions and the cell types were similar to those observed in the controls. However, even in the brains with the more extensive involvement there were fewer pathologic changes than in Group C mice.

Disease in the liver and spleen did not become apparent until the third day after challenge in the Group C mice. Groups A and B, however, had involvement of these organs from their immunizing infection. In Group A only the livers showed pathologic changes. Numerous mature and resolving well-localized granulomas were seen (Fig. 3*A*). There was a tendency over the week of observation (actually the fourth week after infection) for the amount of disease to decrease. The Group B mice showed more extensive pathology even in the first days after the lethal challenge. In the livers,

¶Grayson, J. T.; Menges, R. W.; Altman, P., and Larsh, H. W.: Unpublished observations.

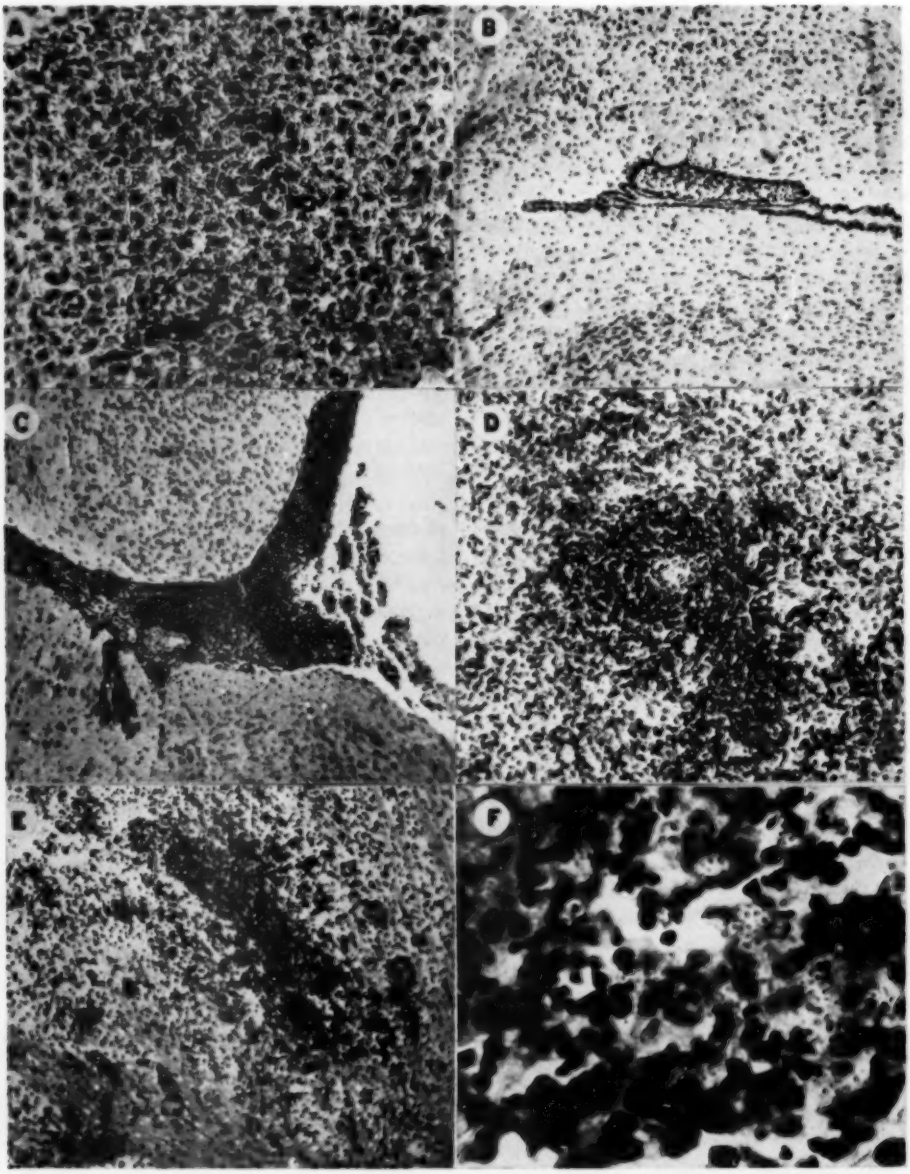

Fig. 3.—Photomicrographs of brain, liver, and spleen sections from control mice and mice immunized by sublethal infection before a lethal intracerebral challenge with 10^6 live cells of the yeast phase of H. capsulatum. All sections are from Experiment 5, and all are stained with hematoxylin and eosin.

A, liver of a mouse which received a sublethal immunizing intraperitoneal injection 16 days previously but was not challenged intracerebrally. Note mature and resolving granulomas; reduced about 1/5 from mag. × 196.

B, brain with minimal round-cell meningitis from an immunized mouse two days after intracerebral challenge; reduced about 1/5 from mag. × 95.

C, brain with extensive meningitis from a control mouse one day after lethal intracerebral challenge; reduced about 1/5 from mag. × 95.

D, infectious granulomas in the spleen of an immunized mouse two days after intracerebral challenge; reduced about 1/5 from mag. × 196.

E, brain with extensive parenchymal destruction seven days after intracerebral challenge in a control mouse; reduced about 1/5 from mag. × 196.

F, intracellular H. capsulatum in great numbers throughout the spleen of a control mouse seven days after challenge; reduced about 1/5 from mag. ×840.

diffuse round-cell infiltration was seen in addition to the mature and resolving granuloma. By two days after challenge localized epithelioid granulomas were present in the spleens (Fig. 3*D*). This was the only group in the five experiments in which granulomas appeared with any frequency in the spleen. Later specimens from Group B showed little change. Irregularities in the amount of involvement were apparent from specimen to specimen. More extensive distortion of splenic white pulp by infiltration of epithelioid cells was seen in some later specimens.

Pathologic changes in the livers in Group C were extensive from three through seven days after challenge. Acute relatively large (100μ) poorly localized granulomas along with varying amounts of diffuse round-cell infiltration were seen. At the end of the observation period the spleens showed complete distortion of normal architecture and massive invasion with H. capsulatum (Fig. 3*F*). Gross enlargement of some of the livers and spleens was noted in the later specimens of both Groups B and C.

The results of the cultural studies are shown in Table 4. The Group A controls had only a low titer of fungi in their tissues. There is not a marked difference between the mice of Groups B and C, but there tends to be more fungi in the previously inoculated Group B mice during the first two days of observation, while there is clearly a greater number of fungi in the tissue of the Group C mice at four and seven days. Since these culture results depend upon pools of three mice, the failure of the fungus titers in Group B to be lower could readily be explained by the irregularity of the protection noted microscopically.

TABLE 4.—*Comparison of the Number of Viable Cells or Cell Aggregates of H. Capsulatum Cultured from Ground Pooled Tissues of the Three Groups of Experiment 5 Where Immunization Was Attempted by Prior Sublethal Intraperitoneal Infection*

Time After Challenge	Log of No. of Fungus Cells per 0.01 Gm. of Tissue								
	Group A (Immunized-Control)*			Group B (Immunized-Challenged)*			Group C (Control)		
	Liver	Spleen	Brain	Liver	Spleen	Brain	Liver	Spleen	Brain
4 hr.	2†	3	0	4	3	6	3	4	4
1 day	2	3	0	5	6	5	5	5	>6
2 days	1	2	0	6	>6	6	5	5	6
3 days	2	4	1	>6	>6	>6	>6	6	>6
4 days	2	3	0	5	6	5	>6	>6	>6
7 days	2	3	1	5	5	6	>6	>6	>6

* See text for details of immunization.
† Each value is for a pool of three mice.

The protection of the brain from intracerebral infection, which occurred fairly regularly following immunization via the intraperitoneal route, was striking compared with the slight diminution in the amount of liver and spleen disease in the same animals. It might ordinarily be expected that sites away from the place of original inoculation would be more easily protected. The present immunization procedures did not prevent dissemination of the fungus from the site of inoculum. The predilection of this fungus organisms to alter the course of sublethal intranasal infections is in agreement with previous cultural studies.[15] It is of interest that sublethal intraperitoneal infection had induced some protection against subsequent intranasal infection.[16]

COMMENT

These studies of the pathology of infections with H. capsulatum following efforts at immunization have confirmed and extended the findings from the mortality and cultural studies previously reported.# Some of the limitations of the present immunization procedures and the irregularity in the immune response were confirmed by the histopathologic studies. The failure of killed

References 14 through 16.

to grow in the reticuloendothelial system has long been known. Perhaps bearing on this phenomenon was the interesting finding in the last experiment that intracerebral challenge of mice already infected intraperitoneally resulted in marked accentuation of the pathologic process in the liver and spleen. This was the only group in which granulomatous lesions regularly developed in the spleen. These findings are consistent with the growing evidence that in some human pulmonary cases with no clinical signs of dissemination the fungus may be present in the blood stream * and granulomatous lesions may be demonstrated in the liver.[21]

One previous report on the pathologic findings in mice infected intracerebrally with H. capsulatum has appeared. Kipkie and Howell [22] injected mice with lethal yeast-phase doses and reported findings similar to that observed in Experiment 5 of the present study.

With the exception of the chronic brain abscesses in the nonimmunized mice, other lesions in both immune and control mice challenged by intracerebral and intranasal sublethal inoculations tended to heal without residual alterations within one to two months. This healing, particularly in the liver and spleen, was different from the pathogenesis of histoplasmosis previously observed in mice by one of us (J. T. G.). Studies employing the mycelial phase of H. capsulatum as the inoculum, even in relatively small doses, resulted in slowly progressive disease, especially in the liver and spleen, and eventual death of a large percentage of the animals.† Probably the most important variation from the conditions of the present experiment was the use of the mycelial phase (spore) of the fungus for inoculum. Also, the fungus isolates used came from human sources, and a different strain of white mice was employed.

The reason that an occasional human infection with H. capsulatum becomes progressive and terminates fatally is poorly understood. The state of natural resistance is known to be a factor. Variation in virulence of the fungus may be a cause. The status of immunity undoubtedly plays a role in the outcome of human infection. The demonstration in these studies of the ability of prior immunization procedures to alter the course of infections offers hope that further animal experimentation may provide a better understanding of immune mechanisms in the deep fungus infections. Such studies might also lead to practical measures of artificial immunization of persons exposed to unusual risk from these diseases.

* References 2, 19, and 20.

† References 23 and 24.

SUMMARY

Serial studies of the pathology of experimental histoplasmosis infections in mice immunized by formalin-inactivated yeast-phase organisms or by prior sublethal intraperitoneal infection compared with the pathology in nonimmunized mice similarly infected are reported. Immunization by either method ameliorates the course of the disease which follows intracerebral challenge. Immunization with inactivated organisms failed to effect the development of disease following intranasal infection.

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Accessory Pancreatic Ducts

Special Reference to the Intrapancreatic Portion of the Common Duct

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The common duct is considered only in passing in English language textbooks of anatomy, histology, and pathology. The impression is left that it is a simple, straight, clean-bore tube, which joins "the" pancreatic duct, passes through a sphincter, and empties at the ampulla into the duodenum. The large volume of contradictory literature concerning this anatomical area attests to the fallacy of this simplified anatomy. In a similar manner, the embryology is considered to be the simple origin of two interdermal anlagen, arising from duodenum which develop into the pancreas and biliary systems. Their continued relation is commonly considered to be purely mechanical; simply owing to gut rotation, the common duct comes to be surrounded by pancreatic tissue through which it must pass to reach the duodenum. The present observations, made from a study of over 400 cases, illustrate the anatomical complexity of the intrapancreatic portion of the common bile duct and its continued functional relationship to pancreas.

MATERIAL AND METHODS

All material used was obtained at the time of autopsy and consisted of 411 specimens of the intrapancreatic portion of the common bile duct. The ampulla and second portion of the duodenum was also studied in many of them. Several cases have been examined by two or more of the methods hereafter described. For the purpose of discussion and for convenience of blocking technique, the intrapancreatic portion of the common duct has been divided into three parts: (1) the upper, (2) the middle, and (3) the transduodenal-ampullary portions. The following methods were used in examining this material:

1. Step sections consisting of a minimum of 4 to a maximum of 319 studies per block of approximately 6 mm. thickness stained with hematoxylin and eosin have been prepared. In the course of this entire study examination by this method has been accomplished in a total of 32 cases. Blocks were taken from Parts 1 and 2 of the common duct and from the second portion of the duodenum in many of them, but from Part 3 in all of them. Included here were specimens from a 5-month fetus, three "premature" infants, and one full-term infant.

2. India ink was injected with gentle pressure and manipulation through the common duct above the pancreas or through the ampulla in 15 specimens. All were then examined grossly in thin sections, and microscopic step sections were prepared and examined in 3 (16 specimens injected and dissected for study of the gross anatomy of the pancreatic and biliary systems are not a part of this report).

3. Vinyl acetate was injected into the common bile duct in eight specimens. After the vinyl acetate hardened, the surrounding tissue was digested away with potassium hydroxide.

4. The common bile ducts of 29 specimens were injected with thin aqueous solutions of barium sulfate and/or potassium iodide. With the assistance of the radiologist, Dr. Gwyllm Lodwick, and his staff, AP and lateral radiographs were made of this material. Two of these specimens were also fixed and sectioned after the radiographs had been made.

5. A finely divided, aqueous charcoal preparation was injected through either the common duct or the ampulla in 32 specimens. After this they were bleached in hydrogen peroxide, dehydrated in alcohol, cleared in benzyl benzoate and methyl salicylate, and examined grossly with low magnification. Five specimens so prepared were also studied in step sections.

6. During the course of 305 autopsies one or more sections of the intrapancreatic portion of the common duct were prepared and examined.

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FINDINGS AND COMMENT

The most satisfactory method of demonstrating the small pancreatic branches of the common duct was that of the injection of aqueous charcoal preparations and subsequent clearing of the specimens. This technique combined with step sections has been most gratifying, both because we have been able to demonstrate clearly the small- and medium-sized branches and also because it enabled us to evaluate more clearly the findings obtained by other methods.

1. *Step Sections Stained with Hematoxylin and Eosin.*—Of the 32 specimens examined by this method, 29 contained small pancreatic ducts either entering the common duct or in such related positions confirmed by injection techniques as to enable us to consider them definitely as branches of the common duct. The 5-month fetus, 2 of the premature infants, and the full-term infant are included in the 29 in which such branches were demonstrated.

Fig. 1.—Vinyl acetate cast from intrapancreatic portion of common duct showing many small pancreatic branches; × 3.

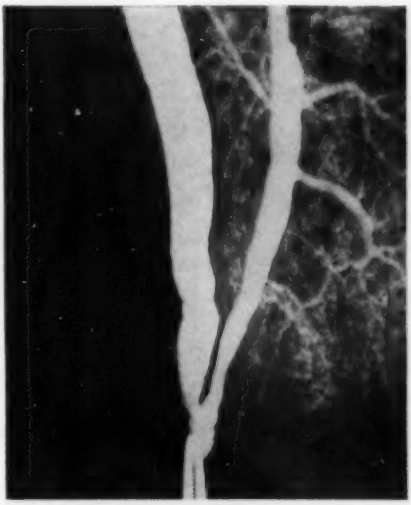

Fig. 2.—Radiograph of common and pancreatic ducts injected with barium sulfate. There is common roughening of both ducts, and small branches can be seen entering common duct in the intraduodenal portion.

2. *India Ink Technique.*—Technical difficulty was encountered in keeping the particulate matter in place during sectioning and staining. In only one of the three sectioned and examined microscopically could definite branches be demonstrated. In 11 examined grossly in cross sections definite branches were found in only 3 instances.

3. *Vinyl Acetate Technique.*—(a) In all eight specimens injected with vinyl acetate a spiny roughening of the intrapancreatic portion of the common bile duct could be demonstrated.

(b) In six specimens small branches of the common duct were present and most numerous in the second and third portions (Fig. 1). The number of branches were of the same order of magnitude as occurred in comparable portions of the main pancreatic duct.

4. *Radiographic Technique.*—The technique was completely unsatisfactory in two. Roughening, comparable in all respects to that of the pancreatic duct, was demonstrated in all of the remaining 27 cases. Small

branches entering the common duct, usually in Part 2 and that portion of Part 3 not occluded by a ligature, were seen in 16, probably in 8, and probably not in 4 (Fig. 2).

5. *Aqueous Charcoal Injection Technique.*—In 30 of the 32 cleared specimens, large, medium, and very small ducts were seen to enter the common duct in the intrapancreatic portion (Fig. 3). The technique was unsatisfactory in two. Branches of the common bile duct were demonstrated microscopically in all five of the specimens examined in step sections.

Fig. 3.—Common ducts which were cleared after ligation about the ampulla and injection with a charcoal preparation. Both large and small pancreatic branches are well shown proximal to the ligatures; × 3.

6. *Findings in Single Sections.*—The findings in single sections from one or several blocks taken at the time of autopsy by several different prosectors do not lend themselves to statistical studies. More than 10%, however, contained branches entering the common duct or in such positions that they could be considered to be such branches. Approximately 20% of sections taken from Part 3 contained such branches.

Small indentations or saccules and the presence of mucous glands are recognized by others as being numerous in the wall of the common duct. The range of opinions regarding these structures is summarized by Burden.[1] He pointed out that they did not penetrate the wall of the duct. He stated, however, that an accidental finding was the presence in several specimens of accessory pancreatic tissue in the wall of the common duct. He did not comment on the possible connection between these and the glands he described. Other authors,[2] starting with the now famous paper of Opie in 1903,[3] also mention the presence of pancreatic lobules adjacent to and in the wall of the common duct. This is actually a frequent finding (Fig. 7) and was encountered in the first case studied. We cannot distinguish visually between the mucous glands and small pancreatic ducts. It is only in serial or step sections or with injection techniques that one can decide whether one is looking at a mucous gland or the terminal portion of an entering pancreatic duct. This same situation exists in the differentiation of the small pancreatic branches and some glands in the ampulla and covering duodenal mucosa. This is reviewed briefly by Loquvam and Russell,[4] who point out that they have been regarded in the past in the ampulla only as mucous glands. These authors report a study of 100 papillae. Small pancreatic ducts were identified in 98%. Many originated in the head of the pancreas, and after penetrating the muscularis, they were found to empty not only into the pancreatic duct but also into the intraduodenal bile duct, directly to the surface of the papilla and through the duodenal mucosa at points removed from the papilla. This was also observed by Opie.[3] We have also shown repeatedly that pancreatic lobules and their ducts do fuse with the common duct in greatest numbers in the region of the intraduodenal and ampullary portion. In that submucous position a corona of small pancreatic lobules and ducts may surround and mingle with the common duct, the pancreatic duct, their common channel or empty directly into the lumen of bowel (Fig. 4).

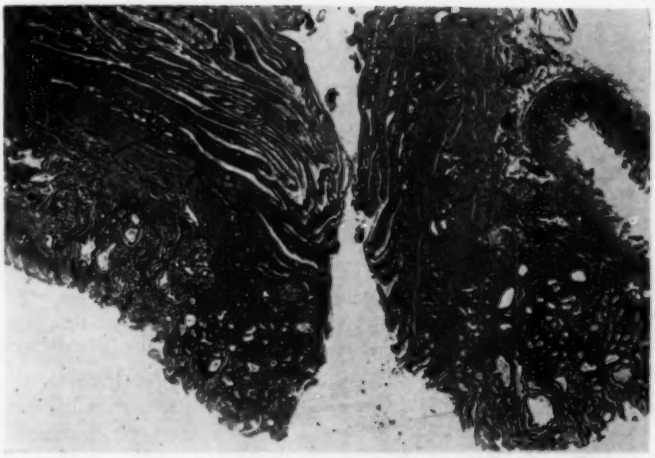

Fig. 4.—The ampulla. The common duct (right) and the major pancreatic duct (left) merge slowly. Smaller pancreatic ducts enter both larger ducts, the common channel, and the lumen of bowel directly. Hematoxylin and eosin; reduced about ¼ from mag. × 13.

The two ducts do not join abruptly but by the fusion of locules, so that frond-like "valves" project into the lumens. In sections, the common duct can usually be distinguished from pancreatic duct by bile staining and greater autolysis. Histologically, however, there appears to be no difference between the two except that the common duct does have a greater applied muscularis (sphincter) in the intraduodenal portion. We were thus not surprised to find in sections prepared from the zones proximal to the union of these ducts that small- and medium-sized pancreatic ducts entered whichever of the large ducts was closest (Figs. 5 and 6). So long as it remains in the pancreas, the common duct receives small branches in the great majority of cases. They arise from lobules in the head and enter the common duct (Figs. 7 and 8), increasing in frequency right down to the tip of the ampulla. In only a few instances have small ducts been demonstrated to join in the upper third by injection techniques, but they are not infrequently seen microscopically.

Just as the small branches join in increasing numbers as the common duct approaches the tip of the ampulla, so also do they increase

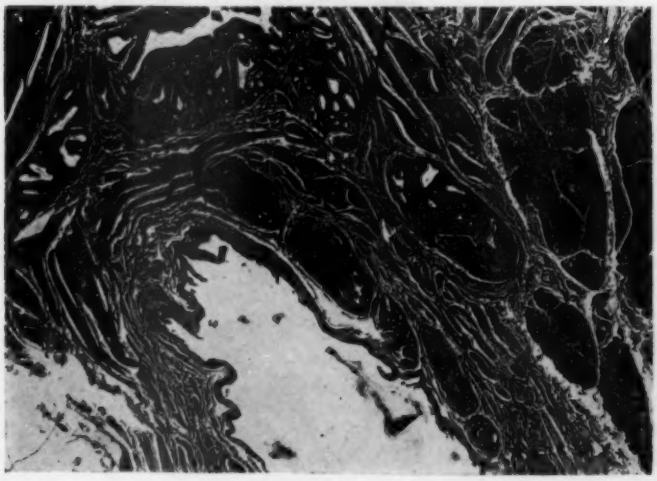

Fig. 5.—The common duct (below) and the pancreatic duct (above) have entered duodenal musculature but have not yet joined. Pancreatic tissue is seen on the right. Note that pancreatic branches join both larger ducts in almost equal numbers. Hematoxylin and eosin; reduced about ¼ from mag. × 40.

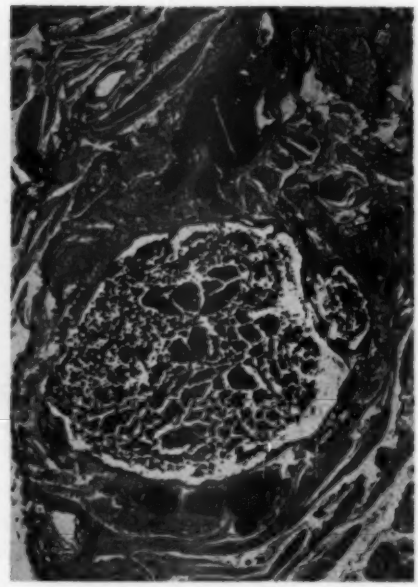

Fig. 6.—The common duct proximal to duodenum. Note the relatively large pancreatic branches entering the common duct. Hematoxylin and eosin stain; × 13.

in size. By all injection techniques, they are thus best shown in the lower portion. It was only late in the course of this work that this was appreciated. A firm ligature had been placed well around the ampulla proximal to a hemostat and had obstructed or prevented the filling of many large pancreatic branches joining the common duct in that location. Many of the illustrations used in this paper are of specimens in which the lower segment has thus been obstructed. (Some of the ligatures are apparent in the photographs; Figs. 1, 2, and 3.)

There are many lobules of pancreatic tissue, continuous with the head of the organ, located within the wall of the duodenum in the second part. Feldman and Weinberg [5] presented a good review of this matter with 31 references. In their own material they identified pancreatic tissue in the various layers of the wall of bowel, principally in the second portion of the duodenum, in 13.7% of 410 consecutive autopsies. This incidence was discovered by gross visualization only. The submucous nodules are usually small, but they may be large and may be visualized on x-ray examination. These were also observed by Opie, in 1903.[3]

On the basis of embryology, it does not seem difficult to understand the incomplete separation of common duct and duodenum from the pancreatic system or to visualize the many variations observed, when careful study is made. The pancreas apparently arises from a segment of entoderm later to be the second portion of the duodenum. The simplified version of the embryology cites two primordia, the ventral and dorsal anlagen, which arise separately but close together. The dorsal bud develops as the body and tail of the pancreas only. The ventral bud, which starts in the direction of the biliary tract, gives rise to a branch which

Fig. 7.—Lower portion of common duct in pancreas. Pancreatic tissue and many ducts, some of which are filled with injected charcoal, are present in the wall. Hematoxylin and eosin; × 13.

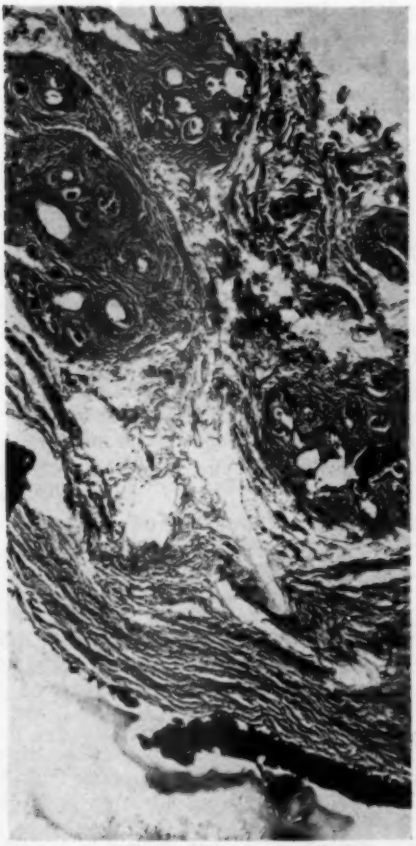

Fig. 8.—Common duct in the upper third of pancreas. The pancreatic branches are partially filled with injected charcoal and were shown in step sections to be continuous with common duct. The transition of ductal epithelium to pancreatic tissue can be seen. Hematoxylin and eosin; × 23.

develops as the head of the pancreas. After fusion with the dorsal bud, the lumen of this portion becomes the main pancreatic duct in most cases. Schwegler and Boiden [6] studied the development of pancreas with special attention to the ampulla in 14 embryos and fetuses. In illustrations, they indicate the presence of small islands of pancreatic tissue seeded in the wall of the duodenum. Zenker (cited by Opie [3]) assumed that pancreatic tissue in bowel wall arose from multiple buds from the intestinal canal. At any rate, the material is present in that location as part of the head of the pancreas in the majority of cases, and in adult life it is found even in the muscularis and submucosa of duodenum. There were points of fusion of head of pancreas and duodenum in all cases (13) in which this portion was examined in step sections. In these cases the head of pancreas and duodenum could not be separated without cutting through pancreatic tissue.

This material is insufficient to evaluate the direct communication of these lobules of pancreatic tissue and the lumen of the duodenum. In the ampulla and adjacent duodenal wall, however, where they are present in great numbers, they can be clearly demonstrated in sections to empty directly into the lumen of the duodenum (Fig. 4). Opie recognized that there was direct communication with bowel.

As in other situations where differentiation occurs over a broad zone, the margins of pancreatic development are not sharp but may often extend in decreasing numbers of nodules proximally as far as the stomach and distally even beyond the ligament of Treitz. Helly (cited by Opie [3]) believed that this pancreatic tissue originated, at a very early period of development, from lateral branches which bud from the duct as it passes through the mesoblastic layer of the intestinal wall. Opie accepted this origin and explained that pancreatic tissue was then carried in both directions by the longitudinal growth of bowel, after which direct communications with the lumen of bowel were established. Ducts passing directly through intestinal mucosa were recognized by Faust and Mudgett.[7] As pointed out by Feldman and Weinberg,[5] this tissue can often be detected on gross examination of bowel; it is a much more frequent finding in microscopic preparations.

In these studies, which included demonstrations of the gross anatomy of the ducts in many, we were impressed, as the volume of literature on the subject also indicates, with the multiple variations in the anatomy of pancreatic ducts in the biliary-pancreatic-duodenal area. Reinhoff and Pickrell [2] state that probably no other region of the body

presents more variations than are found in the relation of the component parts of the biliary tract to one another, to the pancreatic ducts, and to the pancreas, and in the relation of all to the duodenum. The common duct simply grooves the pancreas in a few cases but is completely surrounded in the great majority. The common and the main pancreatic ducts join usually in the wall of the duodenum but occasionally earlier. Occasionally they enter the bowel separately. The matter of "accessory pancreatic ducts," however, is almost a constant. These range from minute lobular branches to large ducts. There are a tremendous number of them in this range of size. They enter the duodenum in the second portion, including especially the ampulla, and they join the common duct in large numbers as it courses through the pancreas. Their size is so variable and their points of termination so frequent in all these locations that the term accessory duct should not be used to apply only to a duct of the dorsal anlage. Loquvam and Russell [4] suggested the term accessory pancreatic ducts for these many branches which they found in the major duodenal papilla. It should include also those which enter the intrapancreatic portion of the common duct and the remainder of duodenum adjacent to the pancreas.

One of the last cases studied in 56 step sections was from a single block obtained from a fetus of 5 months and included the stomach, duodenum, head of the pancreas, and common bile duct, until it reached the cystic duct. Pancreatic tissue in the wall of the duodenum was demonstrated. Three points of entrance of pancreatic ducts into the duodenum were demonstrated in addition to the ampulla. The common duct, as it ascended through the pancreas, received many small pancreatic branches. A cuff of pancreatic lobules was drawn up with it, and in the last section one was present in the wall of the duct just distal to the cystic duct. It is of interest also that a small island of liver tissue was included in the anterointerior portion of the pancreas. It was far removed from the common duct and hepatic branch, the product of the ventral anlage, and reemphasizes the close relation between these early outgrowths from duodenum which differentiate into hepatic and pancreatic tissue.

SUMMARY

The common bile duct and its branches have been studied by several methods in more than 400 specimens, and examination of bowel adjacent to the pancreas was included in many of them. In most specimens, the common duct in its intrapancreatic and intraduodenal portions is joined by many small ducts of pancreatic origin. The common and pancreatic ducts arise together, and as they pass through the head of the pancreas, they remain alike in function and appearance. Many small pancreatic ducts also enter the lumen of bowel directly, particularly at the ampulla and in the second portion of the duodenum. It is suggested that the term "accessory pancreatic ducts" should be extended to include these small ducts which join the intrapancreatic portion of the common bile duct and those which enter the lumen of bowel directly.

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News and Comment

ANNOUNCEMENTS

International Congress on Pathology of Infectious Diseases.—The First International Congress on the Pathology of Infectious Diseases will be held at the Faculty of Medicine and Pharmacy, 8 Avenue Rockefeller, Lyon, France, on May 24, 25, and 26, 1956. The meetings will include discussions of the virus infections of the nervous system, collagen diseases in infections, and the subject of rabies. Further information may be obtained from the Secretariat of the Conference, Institut Pasteur de Lyon, 77 Rue Pasteur, Lyon, France.

American Cancer Society Continues Clinical Fellowships.—The American Cancer Society announces that its program of Clinical Fellowships, begun in 1948, will continue through the institutional year, July 1, 1957-June 30, 1958. Fellowships will be made available primarily to teaching institutions whose postgraduate specialty training programs are approved by the Council on Medical Education and Hospitals of the American Medical Association. For further information address Dr. Brewster S. Miller, American Cancer Society, 521 W. 57th St., New York 19.

PERSONAL

Bertner Foundation Award to Dr. Joseph C. Aub.—Dr. Joseph C. Aub, Professor of Medical Research, Harvard Medical School, and director of medical laboratories at the Collis P. Huntington Memorial Hospital, is the 1956 recipient of the Bertner Foundation Award of the M. D. Anderson Hospital and Tumor Institute, Houston, Texas. The award was presented to Dr. Aub on March 30, after which he presented the Bertner Lecture under the title "Cancer Research Is Growing Up."

Stoneburger Lectures by Dr. Ernest W. Goodpasture.—Dr. Ernest W. Goodpasture, scientific director, department of pathology, Armed Forces Institute of Pathology, Walter Reed Army Medical Center, Washington, D. C., gave the annual Stoneburger Lectures at the Medical College of Virginia, Richmond, on March 21 and 22. These lectures followed a day-time presentation of a symposium on clinical pathology.

SOCIETY NEWS

Maryland Society of Pathologists.—The following is a list of the newly elected 1956 officers for the Maryland Society of Pathologists, Inc.

Dr. Tobias Weinberg, President
Dr. E. C. H. Schmidt, Vice-president
Dr. Paul F. Guerin, Secretary-treasurer
Dr. L. L. Ashburn, Councillor
Dr. John A. Wagner, Councillor
Dr. Tobias Weinberg, Councillor to ASCP

Meeting of Joint Committee on Aviation Pathology.—The Armed Forces Institute of Pathology was host to the first formal meeting of a Department of Defense-sponsored joint committee on aviation pathology, on Feb. 7. Medical officers from the three U. S. armed services were joined by observers from the United Kingdom and Canada as the groundwork was laid for an organization interested in pathology as applied to aviation and flight safety. The group will collect information on the correlation between pathological evidence and causative factors of jet aircraft accidents.

International Symposium on the Diencephalon.—The International Symposium on the Diencephalon was held in Milan, Italy, May 3, 4, and 5.

Books

Cancer Cells. By E. V. Cowdry, M.D., Director, Wernse Cancer Research Laboratory, Washington University, St. Louis. Price, $16.00. Pp. 677, with 137 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia, 1955.

In this book Cowdry has attempted to focus attention on the manifold activities and properties of the cell as the unit of neoplasia. The author, a distinguished cytologist, has long been interested in cells, including malignant cells. Such an interest has made the book particularly valuable because of its departure from the conventional consideration of neoplasms at the descriptive level.

The twenty chapters of the book deal with such aspects as definitions, growth of cancer cells, cytoplasmic and nuclear differences between normal and malignant cells, chemical properties of cancerous tissues, carcinogens, comparative biology of tumors, problems of mutation, heredity and susceptibility, modifying factors in cancer development, etc. Obviously no single author can hope to cover such a vast field adequately, and this fact Cowdry freely admits. Nevertheless, his interests have carried him far, and his fruitful career testifies to his competency in presenting a point of view which should stimulate workers in this field in many ways. The bibliography is extensive, the printing and illustrations are of high quality, and the index has been carefully arranged. It is a book which will be helpful to all concerned with the problems of oncology, presenting as it does both an enormous amount of factual material and a thoughtful evaluation of the many perplexing questions demanding satisfactory answers.

Polycythemia. By John H. Lawrence, M.D., D.Sc., F.A.C.P. Price, $5.50. Pp. 136, with 38 illustrations. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1955.

This monograph by Lawrence deals with the analysis of two hundred thirty-one patients with polycythemia vera, based on the author's experience. Included, also, are studies of seventy-two patients with secondary and relative polycythemia, in which the relationship of these to polycythemia vera is discussed. The emphasis throughout is on physiology, diagnosis, and treatment, with the inclusion of several typical case histories illustrating particularly the value of radioactive phosphorus in the treatment of polycythemia vera. Anyone interested in having a compact summary of the problems of these diseases will find here, in convenient and brief form, the analysis by one of the foremost authorities in the field.

One in Six: An Outline of the Cancer Problem. By I. Hieger, D.Sc. Price 12s 6d. Pp. 80, with 15 illustrations. Allan Wingate Ltd., 12 Beauchamp Place, London SW 3, 1955.

This booklet by Hieger represents an attempt to interpret the main facts and ideas about cancer in terms of modern concepts. Hieger was a member of the original team, under the leadership of Kennaway, which revealed many of the carcinogenic hydrocarbons in tar. The book is dedicated to Beatrice, a little female mouse of the C57 black strain. The title refers to the fact that in England and Wales one death in six at the present time is due to cancer. The author discusses many of the achievements of cancer research, the various theories regarding cancer; and he devotes a chapter to the relation of lung cancer to smoking and smoke. In the epilogue he draws two main conclusions:

"1. Some forms of cancer could be largely avoided by hygienic measures—by changing the technique of living. There can be little doubt that other forms, also, will on further enquiry be shown to be due to environmental causes and could be dealt with in the same way. Only intensive study on a global scale and nothing less than a truly international health service could prove adequate to the task.

"2. We are still a long way from understanding the causation of most of the principal forms of cancer.

"No-one can forsee if the prevention of some forms, at least, of cancer will become feasible before or after pure research reveals the mystery of the cancer cell. At the present moment it is the practical ideas for the prevention of cancer which seem to be in the lead."

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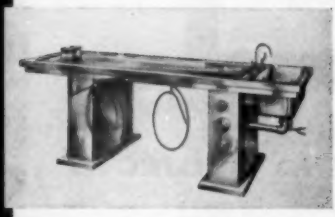

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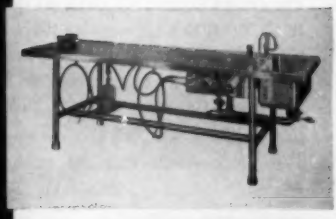

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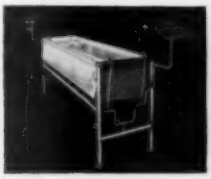

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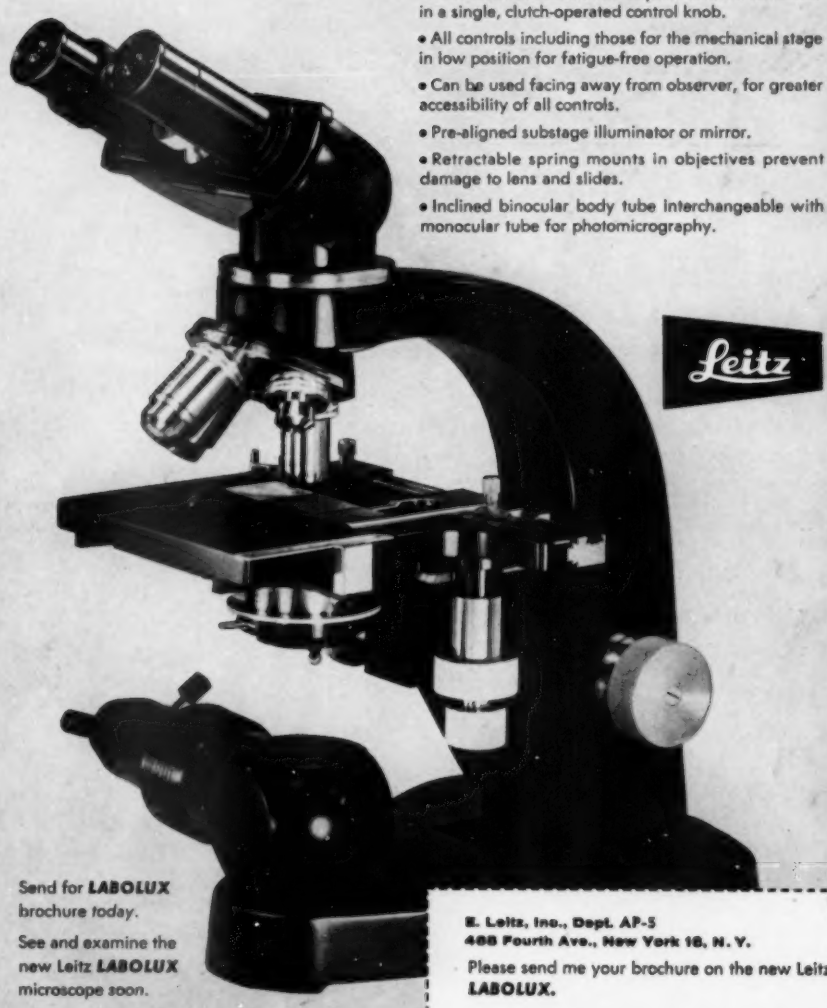

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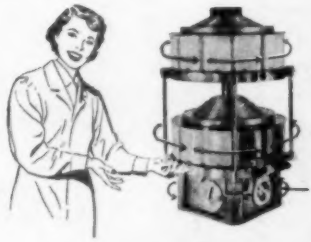

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